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PSP Cover Sheet

Proposal Title: Biological Control of Saltcedar (*Tamarix spp.*) and Giant Reed (*Arundo donax*) in the Cache Creek drainage.

Applicant Name: Raymond I. Carruthers

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Amount of funding requested: ca. $ 250,000/ year for 4 years and a total request of $1,042,885

Indicate the Topic for which you are applying (check only one box).

☐	Fish Passage/ Fish Screens	☒	Introduced Species
☐	Habitat Restoration	☐	Fish Management/ Hatchery
☐	Local Watershed Stewardship	☐	Environmental Education
☐	Water Quality		

Does the proposal address a specified Focused Action? yes no

What county or counties is the project located in? Yolo, County although it relates to wider areas.

Indicate the geographic area of your proposal (check only one box):

☐	Sacramento River Mainstrem	☐	East Side Trib:
☐	Sacramento Trib:	☐	Suisun March and Bay
☐	San Joaquin River Mainstem	☐	North Bay/ South Bay
☐	San Joaquin Trib:	☐	Landscape (entire Bay-Delta watershed)
☐	Delta:	☒	Other: Cache Creek

Indicate the primary species which the proposal addresses (check all that apply):

☐	San Joaquin and East-side Delta tributaries fall-run chinook salmon		
☐	Winter-run chinook salmon	☐	Spring-run chinook salmon
☐	Late-fall run chinook salmon	☐	Fall-run chinook salmon
☐	Delta smelt	☐	Longfin smelt
☐	Splittail	☐	Steelhead trout
☐	Green sturgeon	☐	Striped bass
☐	Migratory birds	☐	All chinook species
☒	Other:	☒	All anadromous salmonids

Specify the ERP strategic objective and target(s) that the project addresses. Include page number from January 1999 version of the ERP Volume I and II:

This proposal relates to various objectives and targets as many species are impacted by the ecosystem changes caused by the target invasive species Tamarix spp. and Arundo donax. However, this project most closely corresponds to Goal 5: Introduced Species, Objective: Halt the introduction of invasive aquatic and terrestrial plants into Central California (page 43). It also is linked with Goal 2: Ecosystem Process and Biotic Communities, through several objectives that help maintain water flow (page 41), and with objectives in Goal 1 Endangered Species (page 38).

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Indicate the type of applicant (check only one box):

☐	State agency	☒	Federal agency
☐	Public/ Non-0profit joint venture	☐	Non-profit
☐	Local government/ district	☐	Private Party
☐	University	☐	Other:_____.

Indicate the type of project (check only one box):

☐	Planning	☐	Implementation
☐	Monitoring	☐	Education
☒	Research		

By signing below, the applicant declares the following:

- The truthfulness of all representation in their proposal;
- The individual singing the form is entitled to submit the application on behalf of the applicant (if the applicant is an entity or organization); and
- The person submitting the application has read and understood the conflict of interest and confidentiality of the proposal on behalf of the applicant, to the extent as provided in the Section.

RAYMOND L. CARRUTHERS
Printed name of applicant

Signature of applicant

Signature of USDA-ARS Authorizing Area Official

Title Page

Title of Proposal: Biological Control of Saltcedar (*Tamarix spp.*) and Giant Reed (*Arundo donax*) in the Cache Creek drainage.

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Type of Organization and Tax Status:

Federal Research Agency (tax exempt)

Tax Identification number: 4716-00000

Biological Control of Saltcedar (*Tamarix spp.*) and Giant Reed (*Arundo donax*) in the Cache Creek drainage.

Executive Summary

Saltcedar and giant reed are two of the most destructive exotic invasive plant species that affect riparian areas in California and other states. They both cause significant damage in the areas of interest to CalFed and are continuing to spread at very high rates within and between many riparian ecosystems. These invasive species are known to out compete native vegetation and commonly form monotypic stands. They provide poor habitat for other flora and fauna, including fish as they cause increased siltation of stream and riverbeds and alter channels in ways that eliminate valuable habitats. They are both excessive water consumers, they increase the likelihood of fire, and most importantly, they alter ecosystem dynamics that further favor their growth and development at the expense of other native species. Together, they are called the "deadly duo" and are thought to be the two most serious exotic plant invaders in California riparian habitats. Their presence and expanding populations clearly threaten other aquatic and semi-aquatic organisms including many Threatened and Endangered Species. This proposal seeks funding to support research on the development of biological control technology for these two species. Biological control has been a very effective method in controlling a number of exotic and invasive plant species in the western states including problematic plants such as purple loosestrife. In regard to this proposal, USDA has already identified and tested biological control agents for saltcedar, but not for giant reed.

This proposal is aimed at three primary tasks,

1) conducting biological release and ecological assessments for saltcedar natural enemies and determining weed control impact,

2) conducting foreign exploration and host-specificity testing for natural enemies of giant reed and introducing appropriate beneficial agents, and

3) developing new benefit/ risk evaluations for the use of exotic biological control agents in sensitive environment areas such as along riparian corridors .

A team approach that includes scientists from a number of state and federal agencies is planned within this proposal that links the expertise of several different groups from areas of basic research to full-scale project implementation. The process will be managed by USDA-ARS but with cooperation from USDI-FWS, UC Berkeley, and USDA-APHIS. Two local groups, Team Arundo del Norte and the Cache Creek Conservancy are implementation partners in the proposed program and will facilitate interactions with the local land owners and county governments. The project requests $1,042.885 for a period covering four years. Overall, this project will field test, evaluate and recommend management options for local groups interested in using biological control as part of an integrated approach to managing these invasive plants. **Task 1** of this project will specifically deliver new biological control agents, implement field releases of approved agents, and conduct evaluations of all biological control agents for *Tamarix* spp. that are appropriate to the Cache Creek environment. **Task 2** will conduct foreign exploration for potential biological control agents of giant reed (*Arundo donax*), it will provide the necessary host specificity testing of both *Tamarix* and *Arundo* agents (both overseas and in US quarantine facilities) and will monitor population and community leve responses to biological control implementation, and **Task 3** will deliver benefit/risk assessments technologies for use of these agents that have been developed from a multi-agency perspective.

Project Description

This proposal seeks funding for the development, environmental risk assessment, testing and evaluation of biological control[1] programs for salt cedar (*Tamarix parviflora* and *T. ramosissima*) and giant reed (*Arundo donax*). These invasive plant species are problematic in the Sacramento/ San Joaquin River Delta area and tributaries as well as in many other locations throughout California and adjacent states. These two invasive plants (saltcedars and giant reed) are being evaluated together as they often occur in the same habitats and have been characterized as the "Deadly Duo" (Bell 1999). Individually and in combination, they out compete native vegetation often forming monotypic stands; they provide poor habitat for other flora and fauna; they cause increased siltation of stream and riverbeds and alter channels in ways that eliminate valuable fish habitats; they are excessive water consumers; they increase the likelihood of fire; and most importantly, they alter ecosystem dynamics that further favor their growth and development at the expensive of other native species (Bell 1997 and 1999). Together, they are thought to be the two most serious exotic plant invaders in California riparian habitats (Bell 1999). Further information on the ecosystem impact of these invasive species can be found in the CalFed proposals that were submitted by Team Arundo del Norte and the Cache Creek Conservancy.

In this project, several private, state and federal groups plan to work as a team through all phases of this effort to better characterize the development and use of biological control in a manner that is environmentally compatible and effective in managing these invasive plants. In addition, this proposal is being developed in combination and in cooperation with two other efforts to manage saltcedar and giant reed. One of these activities is being headed by Team Arundo del Norte, and as the name implies is focused on establishing a regional approach to the control of giant reed throughout northern California; and the other group is headed by the Cache Creek Conservancy which is focused on *Tamarix* and *Arundo* removal in the Cache Creek drainage in Yolo County, CA. Both teams will be putting forward CalFed proposals to implement action programs to manage these species in specific areas while the goal of this proposal is aimed at developing biologically-based tools, primarily classical biological controls, for these and other groups to use in addition to chemical control and physical removal of these pest plants.

Although these two invasive plants will both be included in this effort from project initiation, the associated efforts are in different stages of development. The *Tamarix* effort has already had extensive work conducted by USDA overseas labs and in US quarantine facilities. Based on this work, two biological control agents have been approved by the California Department of Food and Agriculture (CDFA), the USDA-Animal and Plant Health Inspection Service (APHIS) Technical Advisory Group and the US Fish and Wildlife Service (FWS) for environmental release at selected sites. The Cache Creek drainage in Yolo County is one of the sites that was granted approval for agent release in the summer of 1999 (see attached letter from FWS dated 12/28/98 (Appendix A), DeLoach and Gould 1998). Final regulatory approval for the field release is expected this spring when the National Environmental Policy Act (NEPA) documentation is to be completed by APHIS. Since these releases have already been approved

[1] Biological control as defined in this proposal, is the use of co-evolved natural enemies (primarily insect herbivores) that are collected from the sites of origin of the exotic pest plant. After extensive host-specificity testing and regulatory approval, these agents are released into the environment where they are expected to establish, increase in population size, and then impact the target plant through their feeding activities. This is commonly called classical biological control, an approach that has provided effective management of many exotic invasive plants throughout the world in both a highly sustainable and economically efficient manner.

by the CDFA under their biological control permitting authority, no further California Environmental Quality Act (CEQA) approvals are required.

Research on *Tamarix* biological control will focus on experimental field release, agent establishment, biological characterization of the agents under North American conditions, their impact on individual *Tamarix* plants, and the population and community level affects produced in the local flora. A detailed prospectus has been developed by USDA (DeLoach and Gould 1998) that outlines a three-phase operation for cage testing, open field evaluation and full implementation of approved biological control agents in a seven state area which includes parts of California. We are specifically proposing in this document that Cache Creek be considered as the primary research site in the western US where the impact of these agents would be considered in detail on *T. parviflora* and the associated ecosystem. We are particularly interested in how biological control of *Tamarix* will affect the growth, development and recovery of willows and other native plant species in Cache Creek. We are also interested in the effects of gradual *Tamarix* removal on the exotic invader, *Arundo donax* that is currently spreading throughout the Cache Creek drainage and other adjacent areas in northern California.

It is expected that *Arundo donax* may replace the *Tamarix* that would be removed rapidly through physical removal while the *Tamarix* that is eliminated more slowly through biological control may allow native species to be reestablished more effectively through managed revegetation processes. We are interested in the dynamics of these interactions and in understanding how we can better control the succession of plants in this replacement process. Due to the slow acting nature of biological controls, it is hypothesized that native species may be better able to compete with *Arundo donax* under a biological control program then if the *Tamarix* were to be physically or chemically removed over a shorter time period. Current data suggests that native species can effectively compete with saltcedar if they become established before Tamarix. However, saltcedar, being an aggressive early competitor often colonizes areas prior to willow and cottonwoods. Natural enemies may be able to alter these dynamics through herbivory on saltcedar during early periods of colonization and thus all the native species to be more competitive during this critical establishment period.

To further aid native plant species in competing with these exotics, we will explore developing biological control methods for *Arundo donax* as well. The introduction of herbivorous insect that attack giant reed is also expected to favor the competitive ability of desirable native species over exotic invaders. The *Arundo donax* project, however, is still in early stages of development. Work in this proposal will focus on identifying and testing new biological control agents from the geographic areas of origin of this species. This will include foreign exploration and testing of potential natural enemies using the expertise of two USDA biological control laboratories (the European Biological Control Lab and the Sino-American Biological Control Lab) and cooperators located overseas. Planned explorations will focus primarily on the Indian subcontinent, southern China and other locations in South East Asia. Preliminary assessments have determined that giant reed naturally occurs in these areas where it is commonly found but is not considered problematic or invasive (C. Bossard, personal communication). These two USDA Laboratories will assist in this exploration and will conduct first-tier host specificity testing, once appropriate agents have been identified.

Based on the information collected through biological control agent host-specificity testing, a detailed environmental benefit/ risk assessment will be conducted. In the past, risk assessments

have been primarily based on qualitative assessments conducted as part of the regulatory process. In this proposal, we propose to form a new cross-agency team between USDA-ARS, USDA-APHIS, the USDI-FWS, the University of California, and several private groups such as the Nature Conservancy and others to develop and evaluate both improved qualitative and new quantitative methods of evaluating benefits and risks of introducing biological control agents into US ecosystems. As currently documented, *Tamarix* biological control presents a minimal environmental hazard as no North American native plants are known from the Tamaracae. The Nature Conservancy has stated that:

> "Tamarisk is probably the most suitable of "ecological weeds" for investigation of biocontrol. It belongs to a family not native to North America and has only marginal economic use. Its costs, in terms of floodplain management and water consumption, are high, and as will be discussed below, biocontrol agents may be available. However, the U.S. Department of Agriculture must be lobbied to undertake the long and expensive task to developing a biocontrol strategy. Since tamarisk is not an agricultural pest, THE USDA must be specifically encouraged to commit resources" (The Nature Conservancy, Elements of Stewardship Abstract for Tamarix spp., http://tncweeds.ucdavis.edu/esadocs/documents/tamaram.htm).

Based on an effective lobbying campaign from many quarters, USDA took up the task of locating and testing *Tamarix* agents and thus the first tools for biological control of this exotic pest plant have now been made available.

Building upon several years of on-going USDA activities, this proposal will support 3 tasks:

- **Task 1**- the implementation and evaluation of *Tamarix* biological control in one riparian ecosystem, Cache Creek, Yolo County, CA.

- **Task 2**- the development of a parallel *Arundo donax* biological control program including a population level evaluations on the dynamics of these two invasive species (*Tamarix* and *Arundo*) in a single ecosystem, and

- **Task 3**- the improvement of benefit/risk assessment methods for evaluating the actual use of biological control agents in environmentally sensitive areas.

Implementation of this biological control effort and an assessment of its effectiveness on the target pest species will be linked with detailed environmental monitoring procedures that will further characterize both the positive and negative effects that may be exerted in the local environment. Although no negative effects are anticipated, resent concerns about non-target effects of biological control need to be addressed head-on and with appropriate scientific data.

The USDA Agricultural Research Service (ARS), Exotic and Invasive Weed Research Unit has agreed to serve as the Principal Contracting Agency and Dr. Raymond I. Carruthers will serve as the Principal Investigator and Project Coordinator. Dr. Carruthers is housed at the USDA-ARS Western Regional Research Center that maintains the Agency's largest and most advanced biological control of weeds quarantine facility which will serve as the primary receiving and testing site for all incoming biological control agents for both *Tamarix* and *Arundo*. Dr.

Carruthers will take primary responsibility for both project coordination and for testing, monitoring and evaluating all biological control agent introductions. In addition, a cross-agency team including university and federal plant ecologists, overseas biological control specialists, biological control entomologists, and USDI-FWS IPM specialists will be directly involved with the overall evaluation of all biological control agents, assessment technologies to evaluate their impact, and benefit/ risk characterizations of all agents prior to environmental release. Dr. Tom Dudley of UC Berkeley will serve as the coordinator of plant population and community assessments, Dr. Lloyd Wendel of USDA-APHIS will oversee all quarantine operations and agent redistribution, Mr. Scott Stenquist of the US FWS will provide input from the perspective of a conservation agency, and Alan Kirk of the USDA-ARS European Biological Control Laboratory will provide coordination of the foreign exploration for natural enemies of *Arundo donax* in Europe and Asia.

To summarize this overview,

Task 1 of this project will specifically:

- deliver new biological control agents,
- implement field releases of approved agents, and
- conduct evaluations of all biological control agents for *Tamarix* spp. that are appropriate to the Cache Creek environment.

Task 2 will:

- provide foreign exploration for potential biological control agents of giant reed,
- it will provide the necessary host specificity testing of both *Tamarix* and *Arundo* agents (both overseas and in US quarantine facilities), and

Task 3 will:

- provide benefit/risk assessments for use of these agents that have been developed from a multi-agency perspective and
- will use this information to support regulatory oversight of future introductions.

Overall, this project will field test, evaluate and recommend management options for those interested in using biological control as part of an integrated approach to managing these invasive plants. Such an approach has worked extremely well for other exotic pests including several weed species such as purple loosestrife, tansy ragwort, leafy spurge, Klamath weed and others. This effort will be jointly funded through this Cal Fed proposal and through several cost shared projects including funds from the USDA-ARS, USDA-APHIS, the Bureau of Land Management, and the US Fish and Wildlife Service. This project will work directly with both Team Arundo del Norte and the Cache Creek Conservancy to transfer new technology to local action programs. It will also cooperate with a variety of public and private organizations in other areas to help additional groups use this new technology at locations both in California and in adjacent states where these exotic species are significant pests.

We feel that the entire set of three project tasks (**Task 1**- *Tamarix* biological control, **Task 2** - *Arundo* biological control, and **Task 3**- environmental benefit/ risk assessment) are important to develop in parallel. However, for funding purposes these three components could be separated, if necessary, and only one or more aspects of the project could be funded if inadequate resources are available. It would be a shame, however, to implement a successful biological control program for *Tamarix* only to find that *Arundo* quickly fills its niche rather than the desired beneficial native plant species.

Ecological/ Biological Benefits

The overarching goal of this proposal is to conduct research in support of the development of an effective biologically-based management program for saltcedar and giant reed. The goal relates to various ERP goals, objectives and targets as many species within the affected ecosystems are impacted by the environmental changes caused by *Tamarix* spp. and *Arundo donax*. In that regard, this project most closely corresponds to the CalFed Ecosystems Restoration Project Goal 5: Introduced Species and the Objective: Halt the introduction of invasive aquatic and terrestrial plants into Central California. It also is linked with Goal 2: Ecosystem Process and Biotic Communities: through several Objectives that help maintain water flow, and with Objectives in Goal 1 Endangered Species: several Objectives including those that address migratory birds, steelhead and various salmon, pond turtles, and red-leg/ yellow-leg frogs.

It is hypothesized that biological control will help to reduce the population densities of these invasive plant species to levels where both economic and environmental insults caused by their presence have been eliminated or reduced to an acceptable level. Total eradication of these damaging species (the current approach to management) is estimated to cost well over $100 million and probably is not even feasible due to reinvasions. Biological control offers and alternative approach where host-specific natural enemies reproduce, spread, and damage these pest plants on their own. In this way they can reduce pest populations to below critical action thresholds yielding sustainable pest management at affordable costs. The biological control of purple loosestrife and other exotic weeds provide good examples of how this has been achieved in other similar ecosystems. The three primary subgoals of this proposal include: 1) the long-term sustained management of these two inter-related and competing invasive weed species (saltcedar and giant reed) within a single riparian habitat using biological control technologies; 2) the integration of biological control technologies with other methods of invasive species management; and 3) the development and assessment of new methods to help in improved benefit/ risk assessment for biological control programs in ecologically-sensitive areas.

Our overall hypotheses is that the identification and establishment of effective biological control agents for these two plant pests will be a key factor in reducing their populations in a sustained manner. We also hypothesize that this will allow native species to revegetate and further aid in restoration of other attributes of this and similar ecosystems that are affected by these exotic species. We believe that this reduction of detrimental exotic species and the restoration of native plant species will aid all wildlife in impacted areas, including several important fish species and other threatened and endangered species.

To accomplish these goals, several project objectives have been developed that include a series of specific scientific hypotheses that address a hierarchy of related questions. These hypotheses included detailed questions on the biology and population dynamics of both the target plants and the proposed natural enemies (some of which have to be addressed in the countries of origin where the pests evolved); the interactions of the target plant and natural enemies with the abiotic and biotic environment that help to regulate both temporal and spatial synchronies and population abundance; insect/ host plant interactions including feeding dynamics, plant damage impacts, and host plant growth and reproductive responses; and finally inter-plant plant community interactions such as competition for space, resources and other factors that affect changes in the plant community dynamics. A detailed monitoring plan (65 pp) has been developed by USDA (DeLoach, Gould and Carruthers, 1999, Appendix B) that outlines all

aspects of the general release and monitoring plan for insect/ plant population assessment to be conducted through this program.

Technical Feasibility and Timing

Both *Tamarix* and *Arundo* can be controlled through a combination of physical removal, chemical control applications, and/ or altering discharge regimes to favor native species. These approaches, however, are costly and are short-term in that the duration of control is largely dependent on the regrowth and reinfestation rates within the local area. To be effective over the long run, removal projects need to begin upstream in infested tributaries and continue down toward and through the mainstream course. If this is not done, reinfestation in the lower reaches of any riparian habitat quickly erases costly eradication efforts through rapid downstream movement of vegetative fragments and in the case of *Tamarix*, seed. The Bureau of Land Management cites *Tamarix* removal costs at just over $15,000 per acre based on several years of treatment (Ann Knox, personal communication). The Nature Conservancy, Team *Arundo* and Team *Arundo* del Norte, cite similar figures for *Arundo* removal in both southern and Northern California sites. Although the benefits of this removal are significant, both economic and environmental (e.g. removal and disposal of the cut vegetation) problems arise in attempting to conduct massive removal programs.

Biological control, if successful, may provide a long-term and sustainable method to assist in controlling these problems with a minimum of both economic input and collateral environmental damage (disposal of vegetative waste and little or no non-target damage). Once initial investments have been made in the development, testing, release and redistribution of effective natural enemies, the biological control agents have the potential to increase in number and spread throughout the range of their hosts, even to areas that are practically inaccessible to human land managers. Their population numbers are expected to keep increasing as long as these host plants are available for attack and like other host-specific natural enemies, when the host is depleted, large population levels then recede to lower levels and eventually become in dynamic balance with a reduced level of the host plant population. Although some generalized natural enemies have been used in biological control programs in the past (e.g. *Rhinocyllus conicus* for Musk Thistle), the regulatory oversight process and good biological control practices assure a very low probability of unknown side effects from natural enemies released in this program. It is also often difficult to locate and control small founder populations of such plants in remote upstream areas that are often inaccessible. These area are problematic as they provide a source of continual reinvasion of these aggressive plants.

The introduction of biological control agents for both *Tamarix* and *Arundo* are under direct regulatory oversight by the CDFA, USDA-APHIS and USDI-FWS. Two agents have already been approved by CDFA for potential introduction into California and are awaiting final approval by APHIS through the final steps of the NEPA process. An interagency Technical Advisory Group (TAG) of APHIS recommended that these agents be released into the environment. The US Fish and Wildlife Service has written a letter of concurrence in December of 1998 stating that they agreed that these agents should be released following a detailed plan formulated by USDA-ARS and APHIS. In support of these regulatory clearances, a draft Biological Assessment was developed (DeLoach 1997) for the FWS and a final NEPA EA and FONSI are under final review. Release permits are expected in May of 1999 that will allow releases to be made in seven western states. The Cache Creek drainage of Yolo County, CA was accepted as a release site in the FWS approval letter. Once both CDFA and NEPA

documentation are complete, no other environment regulations need to be met before environmental releases of these agents can be conducted. CEQA requirements are all handled through an exclusionary clause linked to CDFA approval that was granted with the original request to release which was made on USDA-APHIS Form-526.

No other issues are expected to limit the use of these biological control agents in the Cache Creek area of California except lack of funding to implement the project. Implementation funding for each of the 13 proposed release site across the 7 state area where *Tamarix* is a major pest, are being sought jointly though both federal and local funds. Although some of the resources are available to conduct this program in Cache Creek (see cost share information), additional resources are required to accelerate this program to a level where valuable tools could be provided to local groups within a 4 year time horizon. Additionally, no funds are currently available to support foreign exploration efforts for *Arundo donax* or to expand benefit/ risk evaluations using new technology.

Monitoring and Data Collection Methodology

Two insect species, *Diorhabda elongata* and *Trabutina mannipara* have both been tentative approved and are being considered for introduction into North America as biological control agents of saltcedar. *Diorhabda elongata* is from western Central Asia and has what are climatically similar to central and northern California. *Trabutina mannipara*, however, can not survive freezing conditions and thus would not be appropriate for release into the Cache Creek area of Yolo County. In the first year of release, *D. elongata* eggs will be transferred from quarantine into release cages established in the lower reaches of Cache Creek. Two proposed field research sites have been identified, one located on Yolo County Flood Control property near the intersection of Cache Creek with Interstate 505, and the other on private land owned by Jan Lowery near Rumsey, CA (see attached map, Appendix C). A proposed future site has been suggested farther north along the drainage near the confluence of Cache and Bear creeks, however, this will not be used in conjunction with this proposal as additional MOUs need to be developed between the Bureau of Land Management and USDA. Similar programs and BLM funding have been acquired to implement a similar saltcedar biological control effort in the Walker River area of Nevada on *Tamarix ramosissima*. In future years, USDA and BLM hope to expand this activity to several additional locations, including the upper reaches of Cache and Bear creeks.

A detailed insect release and monitoring plan has been established for Task 1 (Appendix B) that will coincide with a larger-scale extensive effort to release and evaluate *Tamarix* natural enemies. However, a more detailed evaluation is proposed for the Cache Creek release site so that we can better understand the mechanisms associated with biological control of this pest and its interactions with other plant species including both beneficial species and other exotics such as *Arundo donax*. This plan will be further outline in a following table. During the first season, the newly released biological control agents will be monitored on a twice a week basis to determine their survival, developmental and reproduction rates within release cages. Population increase and impact on the associated target plants will also be assessed. In addition, important non-target species that have already been tested under quarantine conditions will be placed inside these cages and evaluated for any feeding damage under more natural outdoor conditions. In the second year of study, the cages will be removed and the monitoring will continue to assess *D. elongata* survival, development and reproduction in the open environment as well as dispersal rates along the riparian corridor. In cooperation with the Cache Creek Conservancy, detailed aerial photographic data acquired during *Tamarix* bloom (several years of photos already available) will be evaluated to determine the full extent of the existing *Tamarix* infestation. Both

intensive and extensive ground-truthing of plant population densities will be made at and around the original release sites to provide base-line vegetation information for future comparisons with areas where the biological control agents are released. Control sites will be maintained and monitored in areas adjacent to the release sites until the beetles have spread into these areas. We expect several years of comparative data before beetles spread naturally throughout the entire riparian habitat. If appropriate to maintain uninfested control sites in a restricted area, insecticides could be used to eliminate these beetles from a small area. Detailed monitoring will be conducted throughout the experimental area to estimate stage-specific natural enemy population density and growth, natural enemy survival under differing abiotic and biotic conditions, spatial dispersion, and the impact exerted on the target plant.

In addition, more detailed experimental tests will be conducted to evaluate the plant growth and reproduction rates associated with different densities of *D. elongata* infestation. Actual limitations on plant growth caused by beetle feeding will require conducting controlled experiments with several different densities of beetles over a multi-year period. Several additional biological control agents that affect *Tamarix* are known and several are near regulatory approval. If biologically appropriate, these agents could also be introduced into the Cache Creek area. However, the need for additional agents will be evaluated by the entire multi-agency team and only used if necessary. Our goal is to control these exotic pest plants with the smallest number of natural enemies that are possible to use, thus minimizing unneeded introductions. However, it typically requires a complex of natural enemies to control exotic and invasive pests rather than a single agent.

Foreign exploration and faunistic studies to determine the specific biocoenoses of *Arundo donax* in its areas of origin will require extensive-survey to locate natural populations of giant reed. Following these initial surveys, more intensive studies will then be conducted to evaluate these sites through time to characterize the potential natural enemy fauna on all stages and morphological parts of these plants, and at different times during the growing season. This intensive monitoring is typically conducted by local cooperators in areas where the target plant is found to be naturally growing without dominating other natural vegetation in the area. This work will be conducted under the oversight of two ARS Foreign Biological Control Laboratories that specialize in this line of research, the European Biological Control (EBCL) in Montpellier, France, and the Sino-American Biological Control Laboratory (SABCL) in Beijing, China. EBCL will focus it initial exploration into Pakistan and India while the SABCL will explore southern China and other parts of Southeast Asia. Giant reed is known from this entire region which is thought to be the area of origin of *Arundo donax*. In addition to collecting data on the organisms that attack *A. donax* directly, local cooperators will examine other native and introduced plant species (crops, horticultural plants, etc.) to help determine the ecological host range of natural enemies that affect *A. donax*. Once candidate natural enemies have been identified in the field, they will be colonized and returned to the laboratories in France and China where initial host-specificity testing will be conducted under highly controlled conditions. This is the first controlled experimental step in the evaluation process and will include both choice and non-choice feeding tests. Agents that show high degrees of host-specificity in these tests will then be transferred to the USDA quarantine facility in Albany, CA. Here, they will be further tested against members of a host-plant list that will be developed and approved by Inter-Agency review groups such as the APHIS Technical Advisory Groups, CDFA and the US FWS. As discussed in the Project Description, expanded Benefit/ Risk Evaluations will then be conducted to determine which agents are appropriate to release into the North American environment. This assessment would then be used as the basis for any regulatory evaluation and permit request from the oversight Agencies. Foreign explorations will also be conducted in cooperation with other interested groups in Europe and North America that are also interested in locating natural enemies of the pest plant (e.g. IIBC and Cornell University).

Monitoring and Data Collection Information

Hypothesis	Monitoring Parameters	Data Evaluation Approach	Comments/ Data Priority
Task 1			
Environmental Monitoring	Temperature, moisture (vapor pressure deficit, rainfall, dew formation), solar radiation and wind speed/ direction	Field data loggers with appropriate instrumentation	Necessary for nearly all aspects of the program, high priority
Population Development and Reproductive Rate	Stage specific population density estimates	Visual, stem and sweepnet counts in cages and open field situations	Necessary to assess potential insect establishment and growth potential
Natural enemy establishment	Spring population density estimates following overwintering	Emergence trapping of spring adult populations	Necessary to determine successful establishment
Impact on Individual Plants	Defoliation rate on individual test plants	Combination of consumption evaluation studies and digital analysis of photographic/ scanned plant images	Will require individually manipulated controlled studies to be conducted in parallel with general release assessement
Population Level Impacts	Field level evaluation of Tamarix population level reduction in plant size and vigor linked with decreases in numbers and/ or recruitment	Marked plant assessment of plant growth characteristics and an annual assessment of aerial photographs across the release sites	Will be combined with general monitoring of release site and control site flora.
Community Level Impacts	Field level evaluation of vegetation type and cover through time along fixed transects linked with direct assessments of beetle densities and competition with other plant species	Marked plant assessment of fixed tarnsets and an annual assessment of aerial photographs across the release sites	These effects may not be detectable in the four year period associated with this grant, however, base-line plant data must be collected within the first year of this study for long-term comparison
Task 2			
Occurrence of *Arundo* in Proposed Areas of Origin	Presence of absence surveys in habitats expected to support	Conducted through assessments of botanical records and	First step in locating relevant populations of the target pest and

Task 2 (cont.)	giant reed	through site visits by project personnel and cooperators, detected population will be genetically characterized for comparison with US populations	important in determining if biotypes and or other taxonomic differences may exist between the plant at it origin and the introduced genotype
Characterization of Biocoenoses	Assessment of natural enemies that occur on the target plant in it country of origin and on biologically rated plant species in the surrounding area	Typically conducted through visual monitoring, sweepnet sampling and other labor intensive methods. Also highly dependent upon accurate taxonomic assessments.	High priority in determining if potential natural enemies are available and if they show reasonable degrees of host-specificity under natural conditions.
Open-field host-specificity testing	Target plants of interest (both pest and non-pest plants) exposed to natural populations of natural enemy under field conditions in country of origin	Typically conducted through visual monitoring, sweepnet sampling and other labor intensive methods. Test plants may also be seeded with test insects.	High priority in determining if potential natural enemies are available as this can be done at relatively small costs when compared to quarantine tests
No-choice host-specificity testing	Potential beneficial agents are forced to feed upon non-typical host plant or die of starvation. Provides worst case scenario on host-specificity.	Outlines potential risk by defining the physiological host range of the organisms under evaluation.	Can be done either in the country of host plant origin or under quarantine conditions
US based quarantine evaluation	Highly screened agents are brought into US quarantine where they are further tested under US procedures and oversight to verify host-specificity.	Conducted via both choice and no-choice feeding tests based on a critical host plant list developed by outside cooperators.	Extremely important as the regulatory approval to release potential natural enemies into the environment is based on these data.
Task 3			
Synthesis effort no monitoring required			
Task 4			
Management effort no monitoring required			

Local Involvement

This proposed research on the biological control of both saltcedar and giant reed is being conducted directly in cooperation with local groups or through other groups that provide direct linkage to local property owners, county governments and other local groups. Specifically, we are working with the Cache Creek Conservancy and Team *Arundo* del Norte. Both of these organizations have submitted CalFed proposals (Planning and Implementation Proposals) that mention and support the funding of this Research Proposal in helping to produce new biologically-based technologies to supplement their chemical and physical removal programs. The Cache Creek Conservancy is focused directly on the Cache Creek drainage where their mission is to promote the restoration, enhancement and wise management of the stream environment along Cache Creek from Capay to the Settling Basin. They assist in the management of both private and county lands along Cache Creek and are involved in a number of restoration projects important for the future of this area. Both *Tamarix* and *Arundo* are the two most invasive exotic plant species along the waterway and are one of the largest problems that the Conservancy has in conducting restoration efforts along Cache Creek. Although they are beginning to implement a physical removal program in the area, the cost of removing these two species throughout the entire drainage is prohibitive and thus they face continual reinfestation. They are interested in biological control as it may provide them with another method that they can use to lower the negative impact of these species and that hopefully will reduce the reinvasion of these exotic species through sustained biological pressure on these plants. Dr. Carruthers is currently scheduled to speak to the full membership of the Conservancy in May to discuss the logistics of biological control for both *Tamarix* and giant reed.

Team *Arundo* del Norte works across a much wider area of influence in the Northern California area with the goal of assisting others in obtaining both the knowledge and resources to control *Arundo donax* in many different riparian areas. They work with a variety of local groups including property owners, various state and local government agencies, and non-profit environmental groups to help coordinate the removal of this pest plant. In October 1996, Team *Arundo* del Norte was formed as a way to coordinate giant reed research and control efforts in northern California. The team is comprised of representatives from several public agencies, private organizations, and academia. Among the entities represented in the group are the San Francisco Estuary Institute, the California Department of Water Resources, the Sonoma Ecology Center, the U.S. Environmental Protection Agency, and the California Department of Fish and Game. The team identified several areas of focus including public education efforts, the development of scientific and technical information related to giant reed spread and control, and the need for coordinated efforts related to funding and information exchange between various agencies and local groups. Biological control of *Arundo donax* was one of the group's high priority areas of interest. USDA-ARS became involved with this group in March of 1999 to assist in research on *Arundo* reproductive biology (D. Spencer), and biological control potential (R. Carruthers).

Yolo County Flood Control is also a major cooperator on this project (see attached letter, Appendix D).

Cost

Task	Direct Labor Hours	Direct Salary and Benefits	Service Contracts	Material and Acquisition Costs	Miscellaneous and other Direct Costs	Overhead and Indirect Costs	Total Costs
Year 1							
Task 1 Saltcedar biological control		$ 61,600. 2 Research Technicians	$ 20,000 Resources to UC Berkeley to conduct vegetation surveys	$30,000 Environmental monitoring equipment, field cages, sampling materials, genetic reagents, etc.			$ 111,600
Task 2 Arundo foreign exploration and host-specificity testing			$60,000 Foreign exploration resources for EBCL and SABCL				$60,000
Task 3 Benefit/ Risk Evaluation		$ 56,400 1 Post Doctoral Res. Asst.					$ 56,400
Task 4 Project Management	$ 0	$ 0	$ 0	$ 0	$ 0	$22,800	$ 22,800
First year S request							$ 250,800
Year 2							
Task 1 Saltcedar biological control		$ 64,064. 2 Research Technicians	$ 20,000 Resources to UC Berkeley to conduct vegetation surveys	$ 5,000 Miscellaneous Supplies and Materials			$ 89,064
Task 2 Arundo foreign exploration and host-specificity testing			$60,000 Foreign exploration resources for EBCL and SABCL	$ 20,000 Experimental testing of new agents overseas			$ 80,000
Task 3 Benefit/ Risk Evaluation		$ 58,656 1 Post Doctoral Res. Asst.		$10,000 Economic computational assessment			$ 68,656
Task 4 Project Management	$ 0	$ 0	$ 0	$ 0	$ 0	$23,770	$ 23,770
Second year request							$ 261,490

Year 3							
Task 1 Saltcedar biological control		$ 66,626 2 Research Technicians	$ 20,000 Resources to UC Berkeley to conduct vegetation surveys	$ 5,000 Miscellaneous Supplies and Materials			$ 91,626
Task 2 Arundo foreign exploration and host-specificity testing			$60,000 Foreign exploration resources for EBCL and SABCL	$ 20,000 Experimental testing of new agents overseas			$80,000
Task 3 Benefit/ Risk Evaluation		$ 61,002 1 Post Doctoral Res. Asst.		$10,000 Economic computational assessment			$ 71,002
Task 4 Project Management	$ 0	$ 0	$ 0	$ 0	$ 0	$24,262	$ 24,262
Third year request							$ 266,890
Year 4							
Task 1 Saltcedar biological control		$ 34,645 1 Research Technician	$ 20,000 Resources to UC Berkeley to conduct vegetation surveys	$ 5,000 Miscellaneous Supplies and Materials			$ 59,645
Task 2 Arundo foreign exploration and host-specificity testing		$34,645 1 Research Technician	$60,000 Foreign exploration resources for EBCL and SABCL	$ 20,000 Experimental testing of new agents overseas and US quarantine evaluations			$114,645
Task 3 Benefit/ Risk Evaluation		$ 63,442 1 Post Doctoral Res. Asst.		$ 2,000 publication costs			$ 65,442
Task 4 Project Management	$ 0	$ 0	$ 0	$ 0	$ 0	$23,973	$ 23,973
Fourth year request							$263,705
Grand Total							$1,042,885

Quarterly Budget for Project Year 1

Task	Oct-Dec 99	Jan-Mar 00	Apr-Jun 00	Jul-Sep 00	Total
Task 1	$ 36,600	$ 25,000	$ 25,000	$ 25,000	$ 111,600
Task 2	$ 60,000	$ 0	$ 0	$ 0	$ 60,000
Task 3	$ 14,100	$ 14,100	$ 14,100	$ 14,100	$ 56,400
Task 4	$ 11,070	$ 3,910	$ 3,910	$ 3,910	$ 22,800
Total	$ 121,770	$ 43,010	$ 43,010	$ 43,010	$ 250,800

Cost Sharing(estimated based on current commitments)

Agency	Salaries	Operating Resources	Total
USDA-ARS	$ 45,000	$ 120,000	$ 165,000
USDA-APHIS	$ 25,000	$ 8,000	$ 33,000
US FWS	$ 0	$ 12,000	$ 12,000
UC Berkeley	$ 16,000	$ 0	$ 16,000
EBCL and SABCL	$ 25,000	$ 25,000	$ 50,000
Total	$ 111,000	$ 165,000	$ 276,000

Citations

Bell, C. 1997. The Saltcedar Management Workshop, Proceedings from a Workshop, Rancho Mirage, CA, June 1996, 61pp.

Bell, C. 1999. *Arundo* and saltcedar: The Deadly Duo. California Exotic Pest Plant Council. Proceedings from a Workshop, Ontario, CA, June 1998, 158pp.

Bell, G. 1993. Biology and growth habits of giant reed (*Arundo donax*). *Arundo donax* Workshop Proceedings, Ontario, CA November 1993, pp 1-6.

DeLoach, J. 1997. Effects of Biological Control of Saltcedar on Endangered Species: A Biological Assessment for the US Fish and Wildlife Service, non-published document, 555 pp.

DeLoach, J, and J. Gould. 1998. Biological Control of exotic, invading saltcedar by the introduction of *Tamarix*-specific Control Insects from Eurasia, Proposal to the Endangered Species Office of the US Fish and Wildlife Service, 59 pp.

DeLoach, J, J. Gould and R. Carruthers. 1999. Release and Monitoring Plan for Insect Control Agents for Biological Control of Saltcedar, USDA-ARS, non-published document.

Applicant Qualifications

Dr. Raymond J. Carruthers (Supervisory Research Ecologist, GM-15) is the Research Leader of the Exotic and Invasive Weed Research. Dr. Carruthers has a BS (1975) in Biology from California Polytechnic State University, San Luis Obispo and MS (1978)/ PhD (1981) from Michigan State University in Entomology and Systems Ecology. He has been involved with insect population ecology and biological control of invasive species research for over 20 years. He served on the faculty of the Department of Entomology at Cornell University as the Field Crops IPM Specialist before joining USDA-ARS also at Cornell University. At Cornell, Dr. Carruthers studied the impacts of phytophagous insects on crop plants such as corn, alfalfa and small grains. He also conducted research on the use of fungal pathogens to control several

different insect pests that attacked these crops. While working for USDA at Cornell, Dr. Carruthers was stationed in Boyce Thompson Institute for Plant Research where he focused his research on understanding natural disease epizootiology and related this understanding to the use of pathogens as manipulated biological control agents for insect pests such as rangeland grasshoppers, gypsy moths, leafhoppers, and whiteflies. He served USDA-ARS as a Research Leader in Weslaco, TX working on the biological control of *Bemisia* spp. and most recently as the National Program Leader for Biological Control in Washington DC. Dr. Carruthers has received several citations of merit from USDA-ARS and the Entomological Society of America. He is the author of 100+ publications on insect biology, insect pest management, biological control and other related topics.

Recent relevant publications:

Carruthers, R. I., T. S. Larkin, H. Firstencel and Z. Feng. 1992. Influence of thermal ecology on the mycosis of a rangeland grasshopper. Ecology 73: 190-204.

Carruthers, R. I. and J. A. Onsager. 1993. Perspectives on the use of exotic natural enemies for biological control of pest grasshoppers. Environ. Entomol. 22: 885-903.

Hajek, A. E., T. S. Larkin, R. I. Carruthers and R. S. Soper. 1993 Modeling the dynamics of *Entomophaga maimaiga* epizootics in gypsy moth populations. Environ. Entomol. 22: 1172-1187.

Lacey, L. A, J. J. Fransen and R. I. Carruthers. 1995. Global distribution of naturally occurring fungi of *Bemisia*, their biologies and use as biological control agents. pp. 401-433 in, Gerling and Mayer (Eds.), *Bemisia*, Andover Press. 702pp.

Larkin, T. S., A. W. Sweeney and R. I. Carruthers. 1995. Simulation of the dynamics of a microsporidian pathogen of mosquitoes. Ecol. Modelling. 77: 143-165.

Legaspi, B. C., R. I. Carruthers, and J. A. Morales. 1996. Functional response as a component of dynamic simulation models in biological control: the *Catolaccus*-boll weevil system. Ecol. Modelling 89: 43-57.

Carruthers, R. I. and J. K. Petroff. 1997. Proceedings of the invitational workshop on USDA activities in biological control. USDA-ARS. 1991-01, 109pp.

Carruthers, R. I., M. E. Ramos, T. S. Larkin, D. L. Hostetter and R. S. Soper. 1997. The *Entomophaga grylli* species complex: its biology, ecology, and use for biological control of pest grasshoppers. Mem. Entomol. Soc. Canada 171: 329-353.

Legaspi, B. C., R. I. Carruthers, and T. S. Larkin. 1997. New graphical modeling environments: time to reconsider simulation modeling? Amer. Entomol. 43: 105-116.

Wraight, S. P. and R. I. Carruthers. 1998. Production, delivery, and use of mycoinsecticides for control of insect pests of field crops, pp 233-269, in Hall and Menn (Eds.), Methods in Biotechnology, Vol. 5, Biopesticides, Humana Press.

Wraight, S. P., R. I. Carruthers, C. A. Bradley, S. T. Jaronski, L. A. Lacey, P. Wood, and S. Galaini-Wraight. 1998. Pathogenicity of the entomopathogenic fungi *Paecilomyces* spp. and *Beauveria bassiana* against the silverleaf whitefly, *Bemisia agrentifolii*. J Inverte. Pathol. 71: 217-226.

DelFosse, E. S. and R. I. Carruthers. 1999. Roles of the USDA-ARS Foreign Biological Control Laboratories, Proceedings of the Microbial Biological Control Workshop, Feb. 1999. Orlando, FL (in press).

Wraight, S. P., R. I. Carruthers, S. T. Jaronski, C. A. Bradley, C. J. Garza, and S. Galaini-Wraight. 1999. Evaluations of the entomopathogenic fungi *Beauveria bassiana* and *Paecilomyces fumosoroseus* for microbial control of the silverleaf whitefly, *Bemisia argentigolii*. Biological Control. (in press).

APPENDIX A

United States Department of the Interior

FISH AND WILDLIFE SERVICE
Washington, D.C. 20240

1849-1999

In Reply Refer To:
FWS/TE

DEC 28 1998

Dr. Carl Bausch
Deputy Director
Environmental Analysis and Documentation
Policy and Program Development/APHIS
4700 River Road
Riverdale, Maryland 20737

Dear Dr. Bausch:

This responds to your request of December 9, 1998, for our review and concurrence with Dr. Jack Deloach's proposed experimental release of biological control agents on saltcedar of August 28, 1998. Saltcedar infestations have significantly diminished fish and wildlife habitat ecological value in the Western United States, particularly riparian ecosystems. The Fish and Wildlife Service is supportive of the utilization of appropriate techniques to deal with this non-native invasive species. Biological control is one technique that when developed and utilized with appropriate safeguards can and has in some instances provided a viable cost effective and efficient weed control.

We have reviewed your proposal to release 2 insects (leaf beetle (*Diorhabda elongata*) and mealybug (*Trabutina mannipara*)) on 13 sites in the Western United States to determine the effectiveness of these insects in controlling saltcedar and measure the response from native riparian vegetation after release from saltcedar encroachment. Your proposal contains a number of safeguards that will ensure adequate development of sufficient data to make a determination on the larger-scale (entire saltcedar range) in the future and minimize any potential impacts from this experimental release on the endangered southwestern willow flycatcher (*Empidonax traillii extimus*). Measures such as detailed monitoring of the impacts of the insects on saltcedar as well as on native vegetation, geographical isolation of release sites, and distance from any flycatchers occupying saltcedar stands (at least 200 miles) were incorporated into your proposal.

The Service has considered all of the available information regarding the host specificity of these two insects, the potential impacts to the flycatcher, and the monitoring protocols included in your proposal and concurs with your conclusion that this proposed experimental release will not adversely affect the southwestern willow flycatcher.

We appreciate your conscientious efforts to accommodate the Service's concerns and recommendations in the development of this proposal and we look forward to continue working with you in the development of the large-scale biological control program on saltcedar. The

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potential for the ultimate recovery of not only the flycatcher, but of our western riparian ecosystems will depend in grand part to our combined efforts to control saltcedar and provide for the reestablishment of native riparian species. If you have any questions please feel to contact the Division of Endangered Species (Attention: E. LaVerne Smith or Jim Kraus 703/358-2171).

Sincerely,

GERRY [illegible]

ASSISTANT DIRECTOR

cc: 3012-MIB-FWS/Directorate RF
3242-MIB-FWS/AES RF (2)
452-ARLSQ-FWS/TE (LSmith)
452-ARLSQ-FWS/TE (JKraus)
452-ARLSQ-FWS/TE RF (DTE98-00353)
FWS/TE:JKraus/cgl:12/14/98:703-358-2106:S:\DTE\BRC\AGENCIES\APHIS\DELOACH.LTR

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USDA

United States Department of Agriculture

Marketing and Regulatory Programs

Animal and Plant Health Inspection Service

4700 River Road
Riverdale, MD 20737

December 09, 1998

Ms. E. LaVerne Smith
Chief, Division of Endangered Species
Fish and Wildlife Service
4401 North Fairfax Drive
Arlington, Virginia 22203

Dear Ms. Smith:

The Animal and Plant Health Inspection Service (APHIS), on behalf of the applicant Dr. C. Jack DeLoach from the Agriculture Research Service, is requesting the concurrence of the Fish and Wildlife Service on the revised saltcedar proposal submitted on August 28, 1998.

The proposal is to release a leaf beetle, *Diorhabda elongata*, and a mealybug, *Trabutina mannipara*, at 13 sites in the western United States to reduce the abundance of saltcedar, *Tamarix ramosissima*.

APHIS appreciates your cooperation in this matter. If you have any questions, please give me a call at 301-734-8565.

Sincerely,

Carl Bausch
Deputy Director
Environmental Analysis and Documentation
Policy and Program Development

I-020073

APPENDIX B

RELEASE AND MONITORING PLAN FOR INSECT CONTROL AGENTS:

The leafbeetle, *Diorhabda elongata*, and the mealybug, *Trabutina mannipara*,

for Biological Control of Saltcedar

(Research at 13 Approved Sites During 3 Years)

By:

C. Jack DeLoach[a], Juli R. Gould[b] and Ramond I. Carruthers[c]

Reviewed by Insect Monitoring Sub-Committee

Saltcedar Biological Control Consortium

7 April 1999

[a]Research Entomologist, Grassland, Soil and Water Research Laboratory, USDA-Agricultural Research Service, 808 E. Blackland Road, Temple, TX 76502

[b]Supervisory Entomologist, Phoenix Plant Protection Center, USDA, APHIS, PPQ, 4125 E. Broadway Road, Phoenix, AZ 85040

[c]Research Leader, Exotic and Invasive Weed Research, Western Regional Research Center, USDA, ARS, 800 Buchanan St., Albany, CA 94710

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RELEASE AND MONITORING PLAN FOR INSECT CONTROL AGENTS:

The leafbeetle, *Diorhabda elongata*, and the mealybug, *Trabutina mannipara*, for Biological Control of Saltcedar[1,2]

(Research at 13 Approved Sites During 3 Years)

I. LIFE CYCLE AND BEHAVIOR OF THE CONTROL INSECTS

A. The leafbeetle, *Diorhabda elongata*, from western China and eastern Kazakhstan.

1. Biology and behavior. Both larvae and adults feed on the foliage of saltcedar, their only known host plant (see Figure 1, Appendix A). Larvae have 3 instars. Mature third instars crawl down or fall from the plant and pupate under or within litter on the soil surface or sometimes ½ to 1 inch deep in loose soil, or in cracks in the soil. The pupae are bright yellow. Pupae may drown if submerged for very long after rains or high water from streams or lakes. Some papers from Asia indicate that the adults overwinter and others report that large larvae overwinter. This beetle is the most consistently common and most damaging natural enemy of *Tamarix* across Asia, and both adults and larvae may completely defoliate the plant. However, high populations and heavy damage are very sporadic in Asia, reminiscent of insects that are only rarely able to escape their own natural enemies. If not attacked by North American parasitoids or predators, we expect widespread and continuous heavy feeding on *Tamarix*. Host range testing at Temple, TX revealed that survival and damage was much lower on *Tamarix* from some locations than

[1]See Monitoring Plan Outline prepared by Juli Gould, Appendix of 28 August 98 "Proposal" to U.S. Fish and Wildlife Service.
[2] Information from rearing in outdoor cages at Pueblo, CO was provided by Debra Eberts, USDI-Bureau of Reclamation, Denver, CO.

from other locations. The released insects may behave similarly, with the insect being more effective in some areas of the U.S. than in other areas. Climate and other factors also may affect establishment and the amount of control in different areas.

2. Life cycle. The entire life cycle in the laboratory at Temple, TX, at a constant temperature of ca. 25°C, required an average 41 days. Generation time outdoors in midsummer required only 20 days. Duration of the different stages was:

Stage	Duration (Days) ($\bar{x}$±SD) (n) (range)	Head Capsule Width (mm)	Notes
Egg	Ca. 7-10		Tan, globular; single or in masses of 2-20 on leaves
1st Instar Larvae	4.9±1.0(52) (4-8)	0.49±0.03(13) (0.45-0.53)	Ca. 1mm long and yellow at hatching, turning black
2nd Instar Larvae	4.8±1.0(39) (3-11)	0.68±0.04(58) (0.58±0.75)	Gray-black with two indistinct yellow lateral stripes
3rd Instar Larvae	7.4±1.7(28) (3-11)	1.01±0.05(50) (0.88-1.10)	Gray-black with two distinct longitudinal yellow stripes on sides of abdomen, reach ca. 8 mm in length
Prepupa	4.8±1.5(18) (3-8)		Drop to ground, may burrow; adopt C-shaped position
Pupa	7.1±0.8(13) (5-8)		Yellow
Adult pre-oviposition	ca. 3 to 4		
Entire Life Cycle	ca. 41		

3. **Seasonal cycle.** Eggs placed in field cages at Temple, TX, on 29 May produced subsequent generations of adults on 22 June, 10 July, 30 July, and 2 September, for a total of 4 generations during the year. Those placed in cages at Pueblo, CO on 25 June 1997 and 30 May 1998 produced 2 generations a year. One more generation may develop when adults emerge at their natural time, instead of developing from egg shipments later in the season. At Ashghabat, Turkmenistan, overwintering adults emerged in late April.

4. **Critical stages in life and seasonal cycle.** The experience of biocontrol workers indicates that leafbeetles or mealybugs usually transfer readily from one plant to another and establish readily on plants in cages or in the field. However, our experience with these species indicates that establishment may be difficult and may require careful attention to discover interfering factors and to overcome them. Observations by both our overseas cooperators and by us in quarantine at Temple indicate several stages in the life and seasonal cycle where special attention and special techniques may be required to obtain establishment.

a. Oviposition in captivity. The numbers of eggs laid has varied greatly between shipments received from both China and Kazakhstan, for reasons not well understood. This can affect the number of eggs available for release. Sometimes, adults collected from the field in China oviposited well during the 2-4 days before being packaged and hand-carried to the U.S., then oviposition declined drastically after receipt into quarantine at Temple. However, in other shipments, the adults oviposited well for several weeks, especially when very young adults were shipped. In 1998, adults in field cages at Pueblo, CO oviposited well for several weeks.

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b. Neonate larvae. Establishment and the beginning of feeding by neonate 1st instar larvae on the plant was a serious problem in quarantine when egg masses were allowed to hatch on potted plants in cages. Establishment seemed much better in small nylon bags over a branch or when hand-fed in vials. Larvae in outdoor cages seemed to establish more readily than those in quarantine. In 1997 at Pueblo, 65% of the eggs produced larvae that established on the foliage. Eggs that are pulled loose from the bag or foliage on which they were laid have a low survival rate.

c. Pupation. In nature in Kazakhstan, we found numerous pupae on the soil surface underneath litter under infested saltcedar plants. These probably would drown if flooded. Chinese workers are able to control *Diorhabda* by flooding infested areas during the winter.

Rearing methods for pupae in the laboratory has not been perfected. However, if this is needed at some time during the program, the following may serve as a guide.

Large larvae collected near Urumqi, China in 1993 pupated well in plastic boxes in the lab at Beijing. Full grown larvae were placed with foliage in plastic boxes over saltcedar litter ca 1 cm deep, spread over slightly moist soil ca 2 cm deep. Some larvae pupated in the litter, some at the litter-soil interface, and some in the loose soil. Most larvae formed loose cells in the litter and soil that could be picked out or separated by sifting. However, after being hand carried to Temple, very few of those pupae produced healthy adults. We speculate that pupae so produced would produce normal adults if the pupae remained completely undisturbed where they formed

their cells. Full-grown larvae eventually will pupate on filter paper in petri dishes or in nylon bags on the plants but those rarely produce healthy adults if they are disturbed or pulled loose from nylon bags if they pupate there.

The best method known so far for producing healthy adults from large larvae in the field cages may be to remove the bags and let the larvae pupate where they like. However, research by Debra Eberts in field cages at Pueblo, CO indicated that pupae produced in nylon bags survived well and produced normal adults if they were not disturbed. Probably, the soil under the cages should be covered with 1-2 inches of litter from under other saltcedar trees if sufficient litter is not already present, or with 3-4 inches of wheat straw, etc.

d. Overwintering. The overwintering stage is not known with certainty. Different reports from China mention either pupae or adults as overwintering. Maybe both stages may overwinter or maybe it varies in different areas. Probably, the addition of litter from under saltcedar trees or other litter or straw into the cages and maintaining natural moisture levels, may enhance overwintering. We have not yet obtained overwintering in our field cages in the U.S.

B. The Mealybug, *Trabutina mannipara.*

1. Biology and behavior. Mealybugs, in the order Homoptera, have incomplete metamorphosis, where development occurs from the egg stage, through several nymphal stages, to the adult stage. The nymphs resemble the adults except for being smaller; only the adult males have wings (see Figure 2, Appendix A).

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Stages of the manna mealybug, *Trabutina mannipara* (ca. 25°C).[a/]

Stage	Duration	Length x Width (mm)	Notes
Egg	Ca. 7-11 days	0.46 x 0.175	Oval and light yellow-cream, found only inside the ovisacs
1st Instar-Active crawlers	Ca. 1 week	0.5 x 0.2-0.3	Pink to white, dorso-ventrally flattened; highly motile; generally settles among bracts at ends of branches; survive 1-2 days w/o food
2nd Instar-Settled crawlers, w/o white wax	Ca. 1 week	0.7-1.1 x 0.4-0.6	Settled into feeding, motility reduced
2nd to 3rd Instar nymphs - covered with cottony wax filaments	Ca. 5 weeks	3rds: 1.1-1.5 x 0.8-1.0 (♀); 0.9-1.0 x 0.4-0.5 (♂ prepupa)	Nymphs appear to be non-motile; a small droplet of honeydew often occurs exterior to cottony filaments
Small to Medium ♀ Ovisacs	Ca. 3 weeks	Ovisac 1-3 mm in height and diam.	♀ hidden in ovisac; some eggs may be found in medium ovisacs
Mature ♀ Ovisac	Ca. 2 weeks	Ovisac 3-4 mm in height and diam.; adult ♀ 1.2-5.3 x 0.9-3.6	New generation crawlers seen ca. 2 weeks after mature ovisacs, or at 12 weeks from start of development of parent females; large ovisacs averaged 300 eggs each, with a maximum of 785 eggs; females removed from ovisacs lay up to 25 eggs per day; ♀ rotund, pink to blue-gray
Entire life cycle: Ca. 12 weeks			
Winged Adult ♂		1.0-1.2 x 0.3	Emerge from 4th instar ♂ "pupa", greenish, w/3 long caudal filaments

[a/]Data from observations in quarantine at Temple, TX. See Danzig, E.M., and D.R. Miller (1996). A systematic revision of the genus *Trabutina* (Homoptera: Coccoidea: Pseudococcidae). Israel Journal of Entomology 30:7-46 for the published description and more information on the different instars, distribution, host range and other species of the genus.

Trabutina mannipara was redescribed and all stages were illustrated by Danzig and Miller (1996). The species has three nymphal instars. The first-instar nymphs (called "crawlers") crawl rapidly over the plant. Mealybugs damage the plants by sucking out the sap and sometimes also by injecting chemicals into the plant that alter the plant metabolism or kill plant tissue. Several of these groups are either without males entirely, or without males during several all-female generations during part of the year.

Trabutina mannipara is different from most other mealybugs in that the young females secrete a tough, waxy egg sac that encloses her as it grows; when it is complete, she lays her eggs inside the sac. The eggs hatch and after several hours the first-instar nymphs emerge from an orifice in the end of the egg sac and crawl over the plant foliage and to other saltcedar plants. The method of dispersal is not known, but in some mealybug species the first-instar nymphs are wind-blown. The second-instar nymphs have shorter legs, move only short distances, and begin to "settle" on the plant foliage, and begin to secrete white, waxy filaments. The third-instar nymphs remain fixed in their "settled" location and produce large amounts of wax filaments that cover their body giving them their white, waxy mealybug appearance. These colonies of nymphs can become very dense, completely covering the plant terminals; they killed several of our potted test plants in the quarantine studies at Temple. With *T. mannipara*, the females are wingless.

Males of the species were unknown to science until found flying around plants in quarantine at Temple during our tests. They are greenish, winged, with 3 long caudal filaments.

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2. Life cycle. The complete life cycle of *T. mannipara* required about 12 weeks in our quarantine greenhouse studies. The duration of the various stages has not yet been measured.

3. Seasonal cycle. We expect *T. mannipara* to produce about 2 to 3 generations a year in the field. In Israel, Prof. Dan Gerling stated that egg sacs with dead females are found in the field after October. The new generation starts to appear in January as scale-like spots on young branches. They are more apparent during February and reach maturity in April or May. Therefore, they apparently overwinter as crawlers or young 2nd-instar nymphs. In Israel mature egg sacs can be collected during the last week in May. These produced crawlers in quarantine at Temple on 25 May. The next generation of crawlers was produced on 23 August (59 days) and a second generation of crawlers on 22 Nov (91 days), for a total of 3 generations a year in the warmest climates.

4. Critical stages in the life-seasonal history. In our experience to date, laboratory rearing of these insects has been difficult during certain stages. Rearing in the field also may be difficult during these stages and may require careful attention to be successful.

a. Neonate nymphs. Transferring first-instar nymphs to other plants has proven to be very difficult in the laboratory, a procedure that was expected to be very easy. In Israel, the technician worked all one summer attempting to transfer nymphs by allowing them to emerge from the egg sacs into a vial, then inserting the vial over a branchlet of growing *Tamarix*, but was never successful. At Temple, we allowed the crawlers to emerge from the egg sacs onto a square

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of black paper so they could be counted, then we placed the paper on potted saltcedar plants; however, of several hundred eggs, only a few established. In both Israel and Temple, when egg sacs with eggs inside were tied to branches of living saltcedar plants the crawlers established relatively easily. A second generation was then produced on the same plants without further manipulation.

b. Tending by ants. In Israel, *T. mannipara* is heavily tended by the weaver ant, *Polyrhachis simplex*. These ants construct a "tube" of loosely woven litter from the flowers etc. around the twigs on which the egg sacs are attached; I have never observed the egg sacs in nature in Israel that were not covered by the trash tubes. I did not observe nymph infestations and don't know if they also are covered. In quarantine at Temple, development through the entire life cycle took place with no ants present.

The need for ants in the field in the U.S., whether native ants will tend them, and the consequences of whether they are tendered or not (or in what manner) are questions that should be carefully observed in these field releases. Predaceous ants (fire ants in southern Texas) and other predators may severely reduce or eliminate *T. mannipara* if they are not tended by ants. They do produce honeydew, which should attract some types of tending ants.

II. SOURCE OF INSECTS

A. *Diorhabda elongata* Leaf beetles.

1. Overseas source. *Diorhabda elongata* are obtained from two locations in Asia: 1) western

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China in Xinjiang province, at several locations between Turpan and Urumqi and from a location north of Urumqi near Fukang, 2) southeastern Kazakhstan, from sites northeast of Almaty. These insects are shipped (or hand carried) to the ARS quarantine facility at Temple, TX or Albany, CA.

2. Production of clean cultures. "Clean" cultures are produced in quarantine that are free of parasitoids, pathogens or other living organisms. Eggs from the adults received are free of parasitoids. Surface-borne pathogens are eliminated by surface sterilization with chlorine bleach or other similar disinfectant.

Internal pathogens are eliminated by saving the eggs, then examining the females that laid them; if the females are pathogen-free, the eggs may be released or used to produce larvae, or adults of the next generation that may be released. Eggs, larvae or adults are suitable for field release. Pupae appear difficult to culture and probably would not survive if released. Clean cultures also may be maintained in outdoor nursery cages if authorized by APHIS-PPQ. Clean cultures from these outdoor nursery cages also may be used for release at the various field sites. However, subsamples from the outdoor nursery cages should be examined for local parasitoids and pathogens and only shipped to other sites if shown to be free of these.

Insects for release should be either hand carried or shipped overnight. Shipping should be in double-walled containers (as specified by USDA-APHIS-PPQ), with fresh food inside. Copies of proper release permits from APHIS-PPQ and Form AD-942 should be included with each

container. The receiver should complete the form and return a copy to the facility that made the shipment. Proper permits must be obtained from the Department of Agriculture in each state before releasing in that state.

B. *Trabutina mannipara* Mealybugs.

1. Overseas source. *Trabutina mannipara* are obtained from beside the Dead Sea in Israel, mostly from *Tamarix jordanis* or *T. nilotica*. Egg sacs of these mealybugs are shipped to the quarantine facility at Temple, TX.

2. Production of clean cultures. "Clean" cultures are produced in quarantine that are free of insect parasitoids, pathogens, predators or other living organisms. The egg sacs received from overseas may be infested with predaceous larvae of a drosophilid fly but none of them attack first-instar nymphs; therefore, emerging nymphs will immediately be separated from the egg-sac cultures, and can be used to start clean colonies.

At present, the neonate nymphs (crawlers) appear difficult to transfer from one plant to another, and later instar nymphs are largely immobile. Therefore, clean colonies will be maintained in the greenhouse until egg sacs with eggs of the next generation are produced. These egg sacs will be hand carried or shipped overnight to personnel at the release sites. Shipping should be in double-walled containers (as specified by USDA-APHIS-PPQ) with proper release permits and completed AD-942. The receiver should complete the form and return a copy to the facility making the shipments.

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Outdoor, caged nurseries of clean cultures have not been produced and to date have not been authorized by APHIS-PPQ. If authorized in the future, insects from such cultures also could be used to establish colonies at the various release sites.

III. RELEASE SITES AND RELEASE CAGES

A. Criteria for Selection of Release Sites.

The criteria were listed in the "Proposal" to Fish and Wildlife Service of 28 August 1998, as follows:

1. Site isolation. A distance of at least 200 miles from the nearest location where the sw-WIFL is nesting in saltcedar, and isolated from such areas by ecological barriers (desert or mountain ranges) with no connecting strips of saltcedar along which the control insects can migrate.[1/]

[1/]When the "Research Proposal" was submitted on 28 August 1998, all proposed sites were further than the 200 mile limit, the nearest area to the New Mexico site being on the lower San Pedro River, AZ. However, recent surveys by the NM Natural Heritage Program in the San Marcial area just north of Elephant Butte Reservoir on the Rio Grande, NM revealed 4 of the 7 nests of the sw-WIFL to be in saltcedar, and 3 in willow. The three New Mexico sites now are less than 200 miles - Bosque del Apache only ca. 20 miles, Holloman AFB ca. 65 miles, and Artesia ca. 165 miles. The Bosque del Apache site has been given up because of being too near to the sw-WIFL. Hopefully, Holloman and especially Artesia will be retained but these issues have not yet been resolved. Both are isolated from the San Marcial area by deserts, mountain ranges, and both are downwind, all factors making dispersal to San Marcial highly unlikely. Also, the value of saltcedars to sw-WIFL nesting at San Marcial is uncertain. Apparently, both willows and saltcedar occur in dense stands, the sw-WIFL is nesting in both, and nests only in willows in other areas of the middle Rio Grande, including probably also at the Bosque del Apache (some territories found in willows). It would appear likely (although unproven) that the sw-WIFL would nest readily in willows at San Marcial if the saltcedar were controlled, with no reduction in total nesting.

2. Sufficient saltcedar. Stands sufficiently dense and extensive that the control insect populations can increase to large numbers and that dispersal can be monitored meaningfully

3. Soil conditions. The leafbeetle, *D. elongata*, probably requires well drained areas. It pupates on the ground under litter and may drown if submerged for more than several hours. The mealybug spends its entire life cycle on the plant, so soil conditions are assumed to be unimportant.

4. Presence of native vegetation. A major objective of the research releases is to determine the degree of natural revegetation of saltcedar infested sites by native plants, especially by cottonwoods and willows, that occurs following biological control. Therefore, the release sites preferably should contain sufficient remnants of those native trees to provide a seed source so that natural revegetation can occur.

5. Climate. Release sites in different climatic areas are needed to determine the climatic range in which the control insects can be expected to establish and increase. Old World distribution indicates that the leafbeetle *D. elongata* will be effective from northern Texas or New Mexico to as far north as saltcedar grows, but may not be so effective in the more southern areas. The mealybug *T. mannipara* is expected to be most effective in the hottest, most southern areas and may not be sufficiently cold tolerant to survive in the northern areas.

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6. Protection. Sites should be located in areas where the released control insects will not be destroyed by insecticides, fire, herbicidal or mechanical treatments that destroy their food supply, or by vandalism. A written agreement will be obtained from the land owner - manager that he will not intentionally apply insecticides, herbicides, mechanical controls, fire, or other harmful procedures to the vegetation in the release area. A standard form will be prepared by the Saltcedar Consortium (?) for this purpose. The cages should be protected from livestock and wildlife (see Figure 3, Appendix A). The larger area within the boundaries of the release site should have at least some areas where the native vegetation is protected from overgrazing or browsing by livestock or wildlife so that the return of native vegetation can occur and can be documented. Cages should be located so they are not visible from public roads or areas commonly utilized by the public. **The specific location of the cages should not be made available to the general public.** This is to prevent unscrupulous dealers from collecting and selling the control agents, to prevent opponents of biological control from destroying the site, or to prevent those wanting control from collecting the control insects and releasing them on their own lands. All of these actions would destroy the site and prevent obtaining the data needed to obtain authorization for further distribution of the control insects or for understanding their control potential and effects in the ecosystem.

7. Accessibility. The release sites require intensive monitoring for at least 2 or 3 years and less intensive monitoring for a longer period. The sites should be reasonably accessible so that monitoring personnel do not have to walk long distances to reach the sites.

B. Location of Release Sites.

Locations of the 13 release sites, and the insects to be released at each site, are as follows:

Location	Land Owner	Insect to be released
Laredo, TX (Rio Grande)	Private	*T. mannipara*
Seymoure, TX (Wichita River)	Private	*D. elongata*
Big Bend National Park, TX	NPS	*T. mannipara & D. elongata*
Artesia-Bitter Lake NWR, NM (Pecos River)	Private, FWS	*D. elongata & T. mannipara*
Holloman AFB, NM (Tularosa Basin)	DOD	*D. elongata & T. mannipara*
Socorro, NM (Rio Grande)	FWS	*D. elongata & T. mannipara*
Pueblo, CO (Arkansas River)	BR	*D. elongata*
Lovell, WY (Big Horn River)	NPS, WY Fish & Game	*D. elongata*
Delta, UT (Sevier River)	BLM	*D. elongata*
Lovelock-Stillwater-Walker River, NV	Private, FWS, Bl	*D. elongata*
Independence, CA (Owens River)	LA Dept. of Power & Water	*D. elongata*
Clearlake, CA (Cache Creek)	BLM Land	*D. elongata*
Hunter Liggett Military Res., CA (Nacimiento Creek)	DOD, FS	*D. elongata*

C. Specifications for Release Cages.

The big cages for the experimental field releases should be large enough to enclose 2 to 4 medium-sized saltcedar bushes and if possible other smaller plants, and tall enough to allow normal growth of the plants. Big cages should be constructed of a sturdy frame covered with 20-mesh plastic screening, anchored securely against the wind, and buried around the edges to prevent escape of the control insects. Big cages in northern areas must be designed to withstand

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snowfall without collapsing. One or 2 big cages should be constructed at each site for initial releases of the control insects, with an additional 1 or 2 reserve cages to escape from leafhoppers. Ten to 20 small, nylon-plastic sleeve-bags will be used to place some of the insects released inside the cages. Cages should have approximately the following specifications:

1. Cage size. Approx. 12 x 12 ft x 8 ft high, to cover 2 to 4 medium sized saltcedar plants plus some smaller ones and tall enough to allow 1 year of shoot growth after being pruned off.

2. Cage frame. Metal or other framework easy to assemble that will support the cage during the most adverse conditions of wind and winter snowfall expected. The cage frames and fences may be installed before the control insects arrive, possibly in May. The annual report by Debra Eberts gives construction details for her 12 x 12 x 6 ft cages (see Figure 3). Also, David Kazmer has provided specifications for his arched-topped cages that are more suitable in areas with snowfall. Recommendations on cage construction are under review and will be supplied in the near future as an addendum to this monitoring plan.

3. Cage material. Approx. 20 x 20 mesh screening with zippered doorway with a velcro sealed flap over the zippers. Bottom of cage should be fastened to a board or sandwiched between two boards and buried 4 to 6 inches deep on all sides and backfilled with dirt to prevent escape or entry underneath the sides. The Lumite Saran® screen cages can be ordered from Lumite Company (formerly Chicopee), Gainesville, GA (Tel. 770-532-9756) or Pak Unlimited, Inc., Narcross, GA (Tel. 770-448-2369) in sizes to fit the cage frames, either square, flat-topped or the

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arched topped styles, with zippered doors.

4. Sleeve-bags. Ten to 20 or more sleeve-bags will be needed at each site. These bags are made of nylon organdy (or similar material) and clear plastic, 2-liter soda-pop bottles. The plastic bottle is cut in half, both ends cut off, and the organdy is glued to both ends of the bottle segment, making a sleeve on both ends. The bags will be slipped over the terminals of branches, the insects placed inside, and the cloth sleeves tied around the stem with string or twist-ties. The advantage over all-organdy bags is that the clear visibility through the soda bottle allows examination of the insects without removing the bag. The sleeve-bags should be covered with 12 X 18 inch sheets of semi-flexible, reflective insulation to prevent rainfall from soaking the bags and their contents. These sheets can be fastened to the foliage over the sleeve-bags with clothes pins (see Figures 4 and 5, Appendix A).

5. Guying. Cages should be securely guyed to prevent collapse or overturning in the wind.

6. Protection from snowfall. Cages should be constructed to withstand or protect against accumulated snowfall. In southern areas with little or no snowfall, ½ or ¾ inch conduit frames and flat tops are acceptable. In northern areas, heavier frames, such as chain-link fence pipes are necessary. Cages used at Pueblo, CO are protected from snowfall by a carport overhead which is removed during the growing season. Cages used in Wyoming are of arched chain-link fence top rail, anchored at both sides, making a quonset-shaped cage that sheds snow and deflects the wind.

7. Protective fence. Cages should be surrounded by barbed-wire or other sturdy fence to prevent damage by cattle or wildlife. If feral hogs inhabit the area, sturdy livestock panels will be needed. The area between the fence and the cage should be kept mowed or mostly free of vegetation; this will discourage rodents and make any attempts to burrow under the cage easily visible. The fence should be constructed of wooden or metal drive-down posts, with 4-6 strands of barbed wire, 5 ft high, braced or guyed at the corners, with a gate for entry, or another construction providing equivalent security. The fence should be set 3 to 5 ft away from the cage on all sides.

8. Signs. Conspicuous signs should be posted on the fence with the words "U.S. Government Property: Do not open, do not enter or disturb fence or cages - for further information contact _____. Weed Control Experiments in Progress, to benefit wildlife, native plants and agriculture for information call Tel: _____". Do not advertise that the cage contains beneficial insects.

9. Litter. Mature larvae of *Diorhabda* pupate in or under litter on the soil surface. This litter may provide needed protection from either high temperature during the summer or extreme cold during winter (especially since snow is excluded from the cage). Unless already present, or if removed for leafhopper control, litter from under nearby saltcedar thickets, or if not available then other leaves, straw, etc., should be placed under plants inside the cage to cover half to three fourths of the soil surface inside the cage to a depth of 4-6 inches. Also, weeds or grass may be allowed to grow but should be trimmed periodically and this litter left inside the cage.

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Litter probably is not necessary for the mealybug, *Trabutina mannipara*, since all stages develop on the plants.

10. Walkways. Walkways should be constructed between or around the plants inside the *Diorhabda* cages by placing 8 to 12" wide boards on concrete blocks, so that examinations can be made without walking on the pupae. Another, perhaps better, option would be to place boards or metal lattice on bricks, 2 x 4's etc. on the ground, leaving more headroom inside the cage. Walkways are not needed in the *Trabutina* cages since all stages develop on the plant.

11. Predators, competitors, mutualists. Predators and competitors have seriously damaged the saltcedar plants and possibly the control insects in our nursery cages during 1997 and 1998. Rodents or insectivorous mammals found within the cages should be captured and held in a freezer until they can be identified locally. Mutualistic ants may be beneficial to *T. mannipara*. Any insects, spiders or other arthropods found inside the cages, other than the biocontrol insects, should be placed in vials of ethyl alcohol and sent to the Insect Monitoring Team or to taxonomic authorities for identification and recommendation for control, if needed.

Insects sampled from within the cages should be collected in a container with 70-75% ethyl alcohol. After sampling is completed, each sample should then be processed to ensure that the insects and spiders arrive in good condition for identification. After several hours, but not more than 12 hours, the specimens should be poured onto some filter paper or tissue (ensure that the specimens remain on the paper and don't float away with the alcohol), and the liquid discarded.

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Large, soft-bodied specimens such as spiders and lepidopteran larvae should be separated out and put into leak-proof vials with fresh 75% ethyl alcohol. The remaining insects can remain on the paper, however remove most of the liquid. The paper with insects can then be wrapped and placed into a specimen tin or similar container, labeled and mailed. Fisher Scientific sells 4 oz tin specimen boxes (cat. #03-490D) in packs of 36 for around $50.

a. *Diorhabda* cages. Before releasing the control insects inside the cages, ensure that predators such as mice, voles and predaceous ants are not in the cage. Ant mounds inside the cage and within the foraging range of the ants should be treated with appropriate insecticides.

b. *Trabutina* cages. Mutualistic ants may tend these mealybugs, as they always do in Israel. This may or may not be important, but may be very important in protecting them from predators. Only fire ants or other known ants that are predaceous on mealybugs should be controlled around the *Trabutina* cages in order to be able to observe the relationship established with native ants.

c. Leafhoppers and spiders. In nursery cages at both Pueblo, CO and Temple, TX, populations of both the exotic *Tamarix* leafhopper (*Opsius stactogalus*) and spiders have increased to very high populations. The leafhoppers completely defoliated the caged saltcedar plants at Temple, and destroyed 90% of the foliage in one of the cages at Pueblo, CO, depriving the *Diorhabda* beetles of their food supply. At Pueblo, in 1998, leafhopper eggs were hatching on 22 June, foliage was significantly damaged by 7 July, and 90-95% of the foliage had been

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killed in one cage by 15 July. Effects within the sleeve-bags are even more extreme and important damage can occur there while the plants in the big cage show little damage; foliage in the sleeve-bags should be examined frequently. The spiders, which probably were feeding on the leafhoppers, probably also preyed on the *Diorhabda* adults and larvae.

We have not been able yet to devise a satisfactory solution to these problems that does not also kill the *Diorhabda* beetles. The best solution we can suggest at this time is that the control insects should be transferred to an adjacent cage when leafhopper or spider populations rise to threatening levels. This method was effective at Pueblo, CO. The use of yellow sticky traps inside the cages could help reduce adult leafhopper populations, but at Pueblo, this was not sufficient to prevent severe damage. The plants in the cages may be infected in the spring from leafhopper eggs in the leaf litter that has fallen to the ground. An attempt at prevention should be made by removing all leaf litter within the cage area, pruning back the saltcedar branches, and spraying the stems with dormant oil before bud development in the spring, and erecting the cage to prevent reinfestation. The effectiveness of this has not yet been demonstrated. Leafhopper eggs likely are also laid in stems of saltcedar.

The most satisfactory method of eliminating or controlling leafhoppers has not yet been determined. Methods being discussed are 1) trim back stems and remove all litter from inside the cage, 2) spray remaining stems with dormant oil, 3) cover the plant (cage) with airtight plastic and fumigate with methyl bromide or phostoxin, etc.

I-020100

12. Number of cages per site. A minimum of two, and better four, cages should be established at each site. One alternate cage will almost certainly be needed for each working cage to escape leafhopper damage. The screen covers should be left off these alternate cages until needed to prevent a buildup of leafhoppers there. A second working cage at each site is desirable to produce more insects for open-field release during the second year or to provide different plants and environmental conditions in case establishment fails in one cage.

13. Size and number of plants in cages. Each cage should cover a variety of saltcedar plants so the control insects can select the best physiological plant variation for their optimum development. For each cage, 2 to 4 medium-sized plants 8-10 ft tall, should be selected (pruned back to a height of 3 to 4 ft to fit inside the 6 ft or 8 ft high cage) plus a variety of smaller plants. Both old foliage and young foliage should be left intact and new sprouts allowed to grow. The pruning should be done during the dormant season to allow the sprouts to re-grow in time for the springtime releases of control insects in the cages. Optimum habitat conditions for the cages are not known at present. The selection of two different habitats at each site (if more than one working cages are used) would help to answer these questions.

14. Location of cage. Cages should be situated within a stand of saltcedar, on sandy, well-drained soil, in an area unlikely to be flooded, and in an area out of sight of the public, or where the public can be prevented from molesting the cage.

I-020101

IV. RELEASE AND MONITORING OF *Diorhabda elongata* IN FIELD CAGES (YEAR 1)

A. Objectives:

1. Establish the control insects in field cages and monitor their populations, behavior and effects on saltcedar and native plants.

a. Document behavior, survival and development throughout the growing season. The duration of each stage, plus the temperature data recorded and the base developmental temperature determined in separate laboratory experiments, will allow calculation of day-degrees required for completion of a generation, and overwintering at each site.

b. Measure net reproduction rate inside the big cage, both inside and outside the sleeve bags. This will be influenced by climatic and edaphic factors and possibly by arthropod competitors and/or predators, which also must be monitored.

c. Document amount of feeding and oviposition (if any) on selected non-target plants placed inside the big cages.

d. Quantify feeding damage to saltcedar by the control agents inside the big cage.

e. Measure dispersal rate of larvae in the big cages, outside the sleeve bags.

I-020102

B. Method of Releasing *Diorhabda* Inside the Field Cages.

The first releases will be made as soon as suitable insects are available, which probably will be during May. We expect to release eggs, but larvae or adults could also be released, especially later in the season. We expect to ship a minimum of 100 or 200 eggs to each site for the initial releases, but if the females in culture reproduce well more could be sent. Additional shipments could be sent to the sites later in the season if available from the laboratory colonies. The insects will be shipped by the fastest means and so that tracking is possible if the package is lost. The insects should be released at the site as soon as possible after being received. Personnel at each site will be notified by telephone or E-Mail several days before the insects are shipped.

1. Releasing *Diorhabda* in sleeve bags inside the big cages. We expect that the initial releases will be of eggs since they ship well and large numbers of "clean" eggs usually can be obtained. Adults and/or larvae also may be shipped to the release sites, especially later in the season.

a. Releasing eggs. Eggs will be shipped still attached to the twigs or to the nylon bags where laid by the adult females. Eggs laid in culture sometimes fall off with handling. The twigs and any loose eggs should be transferred with a camel-hair brush and glued to Nalgene® paper using Elmers glue and a toothpick. By this method, eggs may be counted in the laboratory, and the number written on the paper, before going to the field. These papers with eggs may be laid on the foliage inside the sleeve-bags or securely attached to the foliage (such as with staples or spring-type clothes pins or paper clips) if released outside the sleeve-bags. Place 10 eggs in each sleeve-bag, 5 bags in each big cage. If eggs are plentiful and more than 100 can be shipped

I-020103

to each site, up to 10 sleeve-bags (100 eggs) should be established in each cage.

As soon as the eggs are fastened to the new terminal foliage, the sleeve-bag should by slipped over the terminal and the sleeve tied around the stem. The sleeve bags are ca. 12 inches long and only about this much terminal should be covered by the sleeve-bag. Do not force much larger amounts of foliage into the sleeve bag, as this is likely to both raise the humidity inside the bag and make the larvae more difficult to find.

b. Mark branches for feeding measurement. If 100 eggs are available, select 20 stems that are approximately equal in size and shape. Measure the distance from the tip of the terminal to where the sleeve is to be tied (12 inches) and mark this area of the twig. If a portable leaf area meter is available, measure the amount of foliage from the 12 inch mark to the tip of the branch. Place a sleeve over each terminal. Randomly select half of the sleeves (10) to receive beetle eggs. Place a tag on each branch that records the date, stem number, leaf area (if applicable) and whether or not beetles were placed in the bag. Monitor the larvae, pupae and adults, as per section C that follows. When new adults emerge, remove them from the sleeve and clip the stem off at the 12 inch mark. Collect the control stems also. If you have a leaf area meter, measure the foliage area of all stems (those with and without beetles). Calculate the dry weight of all stems. Also record the number of beetles that survived in each sleeve. We will compare the initial leaf are of each individual leaf and the final combined weights of stems with and without beetles to estimate the amount of foliage consumed in each cage. Knowing the number of beetles that survived in each cage will allow us to estimate foliage consumption on a leaf by leaf

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basis per beetle during its development.

c. Releasing larvae or adults. These stages may be released into the big cages, either free or in sleeve-bags. The sleeve-bags are useful for establishing the larvae on a branch or for obtaining eggs at a specific location. The sleeve-bags may then be removed after 2 or 3 days.

2. Releasing *Diorhabda* free inside the big cage.

a. Releasing eggs. Eggs should be placed on terminals and covered with sleeve bags as in paragraph IV.B.1.a above. The purpose is to prevent eggs from falling from the plant and the larvae becoming lost on the ground, and also to confine the larvae initially to small areas on the branches. Once the eggs hatch, the sleeve-bags should be removed and larvae allowed to move about freely.

b. Marking the branch. The branch should be marked with a ribbon or string where the sleeve was tied. This ribbon should be tied to the nearest side branch, not on the main branch, in case the ribbon itself might interfere with larvae moving down the branch. This locates the starting point for larval dispersal monitoring.

c. Releasing larvae or adults. Larvae received should be released into larger sleeve-bags (10 x 20 in.), up to 30 larvae per bag. After a few days, when they appear to be well established and feeding on the branch, the bags may be removed. This is a precautionary measure to insure that they do not fall from the plant before becoming established. No experiments are planned for

I-020105

these larvae.

Adults received may be released free inside the cage immediately. They should be placed on the foliage rather than being dumped on the ground. They also could be released into the large sleeve-bags for a few days to determine if they survive and oviposit before removing the bags and setting them free.

C. Monitoring *Diorhabda* Inside the Field Cages.

1. Monitoring schedule. The life cycle of *D. elongata* is expected to vary from 20 to 40 days in the field, depending on temperature. Therefore, frequent monitoring will be needed to closely determine its phenology in the field. Twice-weekly monitoring is preferred at the more accessible sites and weekly monitoring is permissible at more distant sites. During the winter, monitoring may be done only monthly, and only to collect the weather data and confirm security of the cages and fences. Monitoring should resume bi-weekly in the spring to record the date of emergence from overwintering quarters and of reproduction. The *Diorhabda* may be released from the cages into nature as described in paragraph VI below. On each date, the following factors should be monitored, and in the indicated manner:

2. Monitoring inside the sleeve-bags.

a. Survival, development, net reproductive rate, length of generation, behavior.

(1) Egg survival. After the eggs have hatched, open the sleeve-bag, remove all eggs (hold a pan underneath to catch any that may fall), place in a vial with the number of the

bag, and return to the laboratory and record the number hatched or unhatched.

(2) Larval survival and development. Record the number of each stage or larval instar present in each sleeve-bag. Note whether dead or alive and behavior. Any manipulation of the larvae should be done with a small, soft, wet brush. The bags may need to be moved to a fresh terminal after some days, if the larvae have eaten a substantial amount of foliage. If the bags are opened, the larvae may drop, so a pan should be held underneath to catch them.

(3) Pupal survival. Allow larvae to pupate inside the sleeve-bags and record the number. **Do not disturb the pupae!** Pulling them loose if they are stuck to the bag or foliage will probably cause them to die. Even manipulating them with a small paintbrush may cause them to be deformed.

(4) Adult survival, oviposition, age-specific fecundity. Adults that emerge from pupae in the sleeves may be used to measure fecundity. Estimate when you expect the adults to begin emerging and begin monitoring the sleeves as frequently as possible. As adults emerge, carefully untie the sleeve at the free end (the end not tied around the branch). Aspirate to remove all adults from the sleeve, being careful not to disturb unemerged pupae. Repeat this procedure for more sleeves until you have 10 adults collected. Establish a new sleeve with these 10 newly emerged adults. Repeat the procedure until all adults have been removed from the old sleeves. More adults will appear over time and they will need to be collected and placed in new sleeves. Collect these adults as often as possible (daily is best if you are close to the site). When there are

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no more adult to collect from a given sleeve, remove the sleeve and count any eggs that were laid (given the 3-4 day pre-oviposition period, few eggs should have bee laid). Also, take leaf area and dry weight measurement of the remaining stem. When the adult beetles have been in the new sleeves for 3-4 days, open the sleeves, remove any dead adults, and move the new sleeve and the adult beetles to a clean branch. Count the number of eggs laid inside the sleeve every time you move the sleeve. Place collected dead adults in a vial, record the bag number and date of death, return them to the laboratory, determine the sex under a microscope, and record that information. Repeat the above procedure until all adults have died.

(5) Monitoring of 2nd and subsequent generations. Select 50 eggs and place 10 in each of 5 sleeve bags. The best procedure is to leave 10 in place on a terminal, cover with the sleeve bag, and remove the remainder to other branches for liberation in the big cage, first covering them until the larvae hatch as in paragraph IV.C.2.a.(1)-(4) above. Repeat monitoring of the 2nd generation as above for the 1st generation. Repeat this procedure for each generation throughout the growing season. After 50 eggs have been so established, the remainder produced by the 1st generation females may be left on the branches; these do not need to be covered with a sleeve-bag until hatching because, since they were laid under natural conditions in the field, they are not expected to fall off.

b. Other insects and spiders. Record any other insects or spiders present in the sleeve bags and remove them if practical and if they are preying on the control insects or damaging the plant. Make subjective estimates of numbers and damage caused, and move the sleeve-bag to a

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different terminal if damage is severe. (See paragraph IV.C.3.g below).

c. Measure Feeding on Saltcedar. Feeding by the larvae should be measured each time the sleeve-bag is moved, but probably only at the end of the larval development period when all larvae have become pre-pupae or have pupated. If populations of *D. elongata* are at a low level during the first generation after release in the cages, measuring feeding damages should not be attempted, to reduce the risk of damaging the insects in the process. In that case, damage measurements could wait until the second generation. The sleeve-bag then should be carefully removed so as not to disturb the pupae. Most larvae probably will pupate in the top of the bag where it is tied to the stem. The sleeve-bag should be cut off before the tie, leaving the tie and pupae undisturbed. The terminal just distad of the tie should be cut off for measurement of feeding. A new sleeve-bag should be placed over the cut terminal, with the tied part of the old bag (with pupae) in place, and including enough foliage for adult food after they emerge. Any pupae found on the foliage of the old terminal, or on parts of the old bag, should be cut off still attached to a tiny part of the old terminal or bag and placed in the new sleeve-bag for emergence of adults. The paired control terminal should also be cut at the ribbon tie, placed in a plastic or paper bag, and both returned to the laboratory, dried, and dry weight of each recorded. The difference between control and the fed-on terminal will estimate the amount of feeding during the larval stage.

The effects of leafhopper feeding (if any) should be differentiated from *Diorhabda* feeding. *Diorhabda* chew the foliage and leafhoppers suck the sap, but the effects may not be easily

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distinguished. (Hopefully, more information on this will be obtained as the season progresses).

3. Monitoring inside big cages (but outside the sleeve-bags). Larvae liberated inside the big cage during the first generation were from eggs placed in sleeve-bags until they hatched; therefore, the larvae begin their development clustered on the terminal 12 inches of the branches. Larvae of subsequent generations will be mostly from eggs laid by adults free in the cage and will not be clustered.

a. Larval survival, development and dispersal (1st generation). Record the number of each instar on the terminals of 5 branches and record the distance from the original location of the sleeve-bag. This will estimate larval dispersal and larval survival and development outside the sleeve-bags. The accuracy of the survival measurement will depend on whether the larvae crawl too far to be found or fall from the plant, factors not known at this time.

b. Population increase of all stages of *Diorhabda*. After egg laying by the first generation of adults is complete, randomly select and mark 10 to 20 50 cm long terminals (including flowers). Count the number of eggs on each terminal. When egg hatch is complete, remove these eggs from the terminals. After egg laying is complete for the next generation, again randomly select 10-20 50 cm-long terminals in the cage (it doesn't matter if the same terminals are randomly selected again) and count the number of eggs on each terminal. The number of eggs in generation 3 divided by the number of eggs in the previous generation will give us an estimation of population increase (net reproductive rate). Use the life table below to

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calculate this.

LIFE TABLE:

Stage (x)	Number dying during the stage (d_x)	Percentage surviving at beginning of stage (l_x)	Number of eggs laid (m_x)
Egg			
Larva			
Pupa			
Adult Female Days 0-3			
Adult Female Days 4-6			
Adult Female Days 4-6			
Adult Female Days 4-6			
Adult Female Days 4-6			
Eggs			

The net reproductive rate is $\sum l_x m_x$ or the number of eggs in generation 2 divided by the number of eggs in generation 1.

c. Measure preference for different parts of the plant. Examine as in paragraph b above and record all stages on a 50-cm section of old growth on the bottom half of the plant. The quality of the old growth will change throughout the season. Also, briefly examine the plant and record where the most larvae eggs and adults occur - on young terminals or old growth; on top, middle or lower parts of the plant; north, south, east and west sides of the plant; on flowers; on

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older plants or young plants in the cage, on the periphery or the interior of the plant, etc. Count eggs or larvae on two 30-cm terminals in each of these categories without disturbing them or removing them from the plant.

d. Document pupation. At the end of each larval generation, examine visually and record the number of pupae found in two transects under a larger tree. Transects should be 25 cm wide and extend outward from the base of the trees for a distance of 2 m. Record pupae found in each 25 cm section from the tree base outward. Pupae are bright yellow and are expected to be found under litter on the soil surface under infested trees. They may form a loose ball of litter around themselves. They may pupate in cracks in the soil or 1 to 2 cm deep in loose soil. Great care should be taken to search for the pupae gently and to cause them the least disturbance possible. Our experience shows that disturbance may cause the pupae to die or that weak adults may emerge. These observations should not be attempted until the 2nd or 3rd generation, and until the beetles are reproducing well, so as not to deplete the population if the observation method reduces pupal survival.

e. Document overwintering. In late winter or early spring, examine 25 cm^2 areas under infested trees and record the overwintering stage of *Diorhabda*, which could be mature larvae, pupae or adults. In the spring, when maximum daily temperatures rise above 10°C (50°F) begin weekly or twice weekly monitoring to detect the first adults or other active stages. Also record bud-break and shoot elongation.

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f. Quantify feeding damage to saltcedar. All damage estimates will include damage by other insects, most probably by leafhoppers. Therefore, an attempt should be made to judge the effects of *Diorhabda* separately from that of the other insects and to estimate the populations of all insect species (see g. below).

(1) Measure damage to the plants at the end of each larval generation, inside the big cages.

(2) Measure dry weight of 10 each 10-cm long lateral terminals cut from plants inside the big cage that represent average damage conditions within the cage. Also, cut 10 similar 10-cm long lateral control terminals from outside the big cage, also measure dry weight. The difference in dry weight between control and insect damaged terminals estimates dry weight consumed by the insects.

(3) Visually estimate "damage categories" that represent damage inside the big cage. Categories are: no apparent damage, 5 to 10% damage, 10-35% damage, 35-70% damage, 70-90% damage, 90-100% damage.

(4) Use of an electronic leaf area meter is being investigated. If this method appears feasible, monitoring personnel at the various sites will be notified.

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g. Estimate population of other arthropods. Our past experience in caging saltcedar, and the work of others (Liesner 1971) indicates that the exotic leafhopper, *Opsius stactogalus,* and various spider species may rapidly increase to very high levels inside cages. The leafhoppers may kill all the foliage on the saltcedar plants, which then will deprive *Diorhabda* beetles of their food resource and cause their death. The spiders probably feed on the leafhoppers but also may prey on the *Diorhabda* larvae and adults. Sometimes, the tiny, white, exotic, scale insect, *Chianapsis eutrusca,* also may attain populations sufficient to kill the foliage and small branches. Other predaceous and phytophagus insects may enter and increase within the cages. While these insects seldom increase to high populations in nature, they can seriously compromise our attempts to establish *Diorhabda* and to monitor its populations and effects on saltcedar in cages. Populations of the arthropods in the large cages should be monitored as follows:

(1) Visually examine the plants in the cage and determine areas with leafhoppers, spiders or other insects.

(2) Sample four terminals each week (or each 2 weeks) that represent population levels in the cage. Sample the insects by shaking 50 cm-long terminals into a sweep net. Then dump the insects and spiders into a transparent plastic bag, collect all insects and spiders with an aspirator (omitting the plant trash and the *Diorhabda*). Remove the *Diorhabda* larvae and adults and place them back on the growing plants. Quickly open the aspirator and dump the insects into a small glass killing jar. The jar can be made with ca ½ inch of plaster of Paris in the bottom, onto which ethyl acetate has been poured and soaked into the plaster, and the bottle

closed with a large cork (not rubber) stopper. After the insects die (a few minutes) they may be left in the killing bottle, or transferred to a vial or small metal sample box, covered with tissue paper, labeled and returned to the laboratory for identification. Immature insects must be rapidly transferred to alcohol or other appropriate fluids according to standard entomological procedures. Alternatively, all insects may be dumped from the aspirator into a vial of 70% ethanol in the field, then those to be pinned should be removed that night and stored dry or pinned for identification, counting, and recording. More than a few hours in alcohol will destroy certain insects (such as leafhoppers) for identification. More detailed insect collecting and preserving techniques will be furnished by the Insect Monitoring Team.

h. Record damage to non-target plants inside the big cage. At each site, non-target species of importance in riparian areas may be established in the big cages for a field host-range test. These species could include willows, cottonwoods, seepwillow baccharis or other species. The plants could be planted in the soil or placed in pots inside the big cage; they must be watered frequently until well established; "Dri water" could be used at remote sites. These species already have been tested extensively in quarantine at Temple, TX but not under field conditions.

On each monitoring date, these plants should be carefully examined visually and the numbers of each stage (adults, eggs, larvae) of *Diorhabda* recorded. If larvae or adults are feeding on the plants the amount of feeding should be recorded. If oviposition or feeding is discovered on these plants, the Chairman of the Insect Monitoring Team of the Saltcedar Consortium must be notified immediately. In the history of biological control of weeds, non-target feeding by the

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control agents sometimes has occurred when the control agents reach high populations, then eat most of the target weed and spill over onto nearby non-target plants. This almost never continues after peak population passes but such an occurrence must immediately be analyzed carefully by the Insect Monitoring Team.

V. RELEASE AND MONITORING OF *TRABUTINA* IN FIELD CAGES

A. Objectives:

1. Establish reproducing and overwintering colonies of *Trabutina* on saltcedar plants in field cages.

2. Document behavior, survival, reproduction, and development throughout the growing season and time required for completion of a generation.

3. Measure population increase between generations.

4. Document dispersal by crawlers inside the cage.

5. Quantify damage to saltcedar inside the cages.

6. Document infestation of and development on non-target plants inside the cages.

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B. Methods of Releasing *Trabutina* in Field Cages.

The first releases will be made as soon as suitable insects are available, which probably will be during May or June. We expect to release egg sacs, but crawlers could also be released, especially later in the season. We expect to ship a minimum of 10 to 15 egg sacs to each site for the initial releases, but if the females in culture reproduce well more could be sent. Additional shipments could be sent to the sites later in the season if available from the laboratory colonies. The insects will be shipped by the fastest means and should be released at the site as soon as possible after being received. Personnel at each site will be notified by telephone or E-Mail several days before the insects are shipped.

1. Release in sleeve-bags. Egg sacs most likely will be shipped for release at the sites. Crawlers may be sent on some occasions, depending on availability and timing of the nursery cultures.

a. Releasing egg sacs. Egg sacs should be released as soon as possible after being received at the release site (either the same day or the next day), before the neonate nymphs emerge. The egg sacs will arrive attached to a small section of stem. This stem with attached egg sacs should be tied to the stem of a terminal branch of a saltcedar plant growing in the big cage, then covered with a nylon-plastic sleeve-bag, and the sleeve tied around the branch. From 3 to 5 egg sacs should insure strong establishment. Establish 5 such sleeve bags in the big cage, each on different branches and some on different plants in the cage. Each sleeve-bag should be marked with a numbered tag.

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b. Releasing crawlers. Sometimes, crawlers may be shipped or hand carried to the sites. These probably will be on growing, potted plants. Past attempts at establishing crawlers has been mostly unsuccessful, but if received, an attempt should be made to establish them. If some crawlers have emerged in the shipment of egg sacs received, these could be released in the field cages also.

Probably, the best way to establish crawlers is as follows: Cut the potted stem with crawlers attached, intertwine it with foliage from a terminal of a plant inside the big cage, tie the entwined branches together loosely with string, and either enclose it in a sleeve bag or leave it unbagged.

2. Releasing Free (Not in Sleeve-bags) Inside the Big Cage. Follow the same procedures as above for either egg sacs or nymphs. Part of the egg sacs received should be released free in the big cages as above, but not covered with a sleeve bag. *If two big cages are available at the* release site, the egg sacs in sleeve bags should be placed in one cage and the unbagged releases made in the other big cage. In this way, dispersal of the crawlers could be measured inside the cage without interferring with the population monitoring of the sleeve-cage, fixed-location monitoring.

C. Monitoring *Trabutina* in Field Cages.

1. Schedule of Monitoring. The life cycle of *T. mannipara* is expected to vary from 30-40 days in midsummer to 90 days or more during the cooler seasons. Therefore, weekly monitoring

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during the first growing season probably will be sufficient to determine its phenology in the field. More frequent monitoring probably is not necessary because a) the crawlers are unlikely to be found, b) settled nymphs become easily visible only after they begin secreting wax, which requires several days, c) and development through the nymphal stage is slow and instars are difficult to distinguish. During the winter, monitoring needs to be done only monthly, to determine the condition of the cage, to download the weather data, and to confirm the condition of the overwintering stages of *Trabutina*. Monitoring should resume each week in the spring to record the date of emergence from overwintering quarters and of reproduction. The *Trabutina* may be released from the cages into nature after overwintering is demonstrated.

2. Monitoring Inside the Sleeve-bags.

a. Development: crawlers to egg sacs. On each sampling date, record the number of crawlers (if they can be found), small nymphs without wax, nymphs with a small amount of wax, nymphs with much wax, small egg sacs, and large egg sacs.

Establish the colonies in sleeves (one egg sac per sleeve), with multiple stems in each sleeve (as many as practical). Remove the sleeve after the crawlers settle. It will be much easier to count and monitor them without the bag and the insects should stay in place. At the end of the generation the number of egg sacs can be counted for a calculation of the net reproductive rate.

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b. Generation time, reproduction rate. These parameters may be estimated for the period from mature egg sacs released, to mature egg sacs of the next generation. Size of egg sacs should be measured to obtain an estimate of numbers of eggs (already known for different sizes of egg sacs). However, losses probably will be so great, and nymphs so difficult to count, that rate of increase will be most closely estimated from egg sacs of each generation, possibly with a weighing factor for sizes of egg sacs. These monitoring and population estimates may be repeated for each generation produced.

c. Other arthropods in the sleeve-bags. Numbers of spiders, leafhoppers and other insects in the sleeve-bags, and notes on the amount of damage to the plant, should be recorded on each monitoring date. In the previous field nursery cages at Pueblo and Temple, large populations of leafhoppers have developed inside the bags that killed all the foliage. Unlike *Diorhabda*, the *Trabutina* cannot be moved to a fresh terminal because only the crawlers are mobile. Attempting to move later stages, either directly or by cutting off sections of stems, will probably kill the nymphs or adults in the egg sacs. At the present time, we cannot suggest a reliable method for preventing damage from leafhoppers. The best (but untried) method may be to remove the sleeve-bag, catch the leafhopper adults with an aspirator, brush off or mash the nymphs, and either replace the bag or leave it off.

d. Transfers after first generation. Past experience in the quarantine greenhouse at Temple (on uncaged, potted plants) indicates that more than one generation probably cannot be maintained in a sleeve-bag. If several 2nd generation egg sacs are produced, the number of

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nymphs produced probably would rapidly kill the terminal and the nymphs would also die. However, since both nymphs beyond the crawler stage and adults are immobile, little is to be gained from holding them in sleeve-bags. A better concept is to remove the sleeve-bags as soon as the small, white nymphs become visible, and continue to monitor the population that, although uncaged, will remain fixed at the same location on the terminal. To measure population increase, all mature egg sacs produced by the 1st generation nymphs should be transferred to new sleeve-bags, a few in each bag, and recorded all together for the 2nd generation total. If many egg masses were produced by the 1st generation nymphs, then a subsample of 5 or 10 egg sacs could be used in a similar manner. Again, the sleeve-bags should be removed when the small-white nymphs become visible, and monitoring of the 2nd generation should continue as described above for the 1st generation.

3. Monitoring in big cages (but outside the sleeve-bags). The nymphs free in the big cage will have developed from crawlers that dispersed from the egg sacs tied to the saltcedar terminals; they may be restricted to a small area on the original release branch or they may have dispersed throughout the cage. In Israel, egg sacs are found mostly on twigs 1/8 to 1/4 inch diam; in quarantine at Temple, nymphs were found mostly on tender, young terminals. Males have only been found in quarantine at Temple, TX (see Section I.B.1). Observe and record their presence (if any) in the cages.

a. Survival, dispersal and development of nymphs. Visually examine all (or most) of the foliage inside the big cage to locate areas where the white nymphs have established. Then,

permanently mark 10 representative 20-cm-long terminals and record weekly the number of small and large nymphs, and small and large egg sacs. Attempt to determine if neonate nymphs are, or can, crawl through the 20 x 20 mesh screening of the big cage and become windblown or otherwise escape from the cage. Observe any attack by predators or other mortality factors.

b. Preference for different parts of the plant. Record the part of the plant (top, bottom, foliage, flowers, stems, north, south, east, west, old foliage-stems, new foliage-stems) on which nymphs and egg sacs are found.

c. Overwintering. Document the stage of the mealybugs and the location on the plant where overwintering occurs. Resume weekly monitoring in the spring to document the date of emergence from overwintering quarters and the beginning of reproduction.

d. Mutualism between *Trabutina* and ants. In Israel, egg sacs are always tended by ants, which feed on honeydew produced by the mealybugs and that build protective webs of saltcedar litter over the mealybugs. In quarantine at Temple, *Trabutina* developed well through 2 generations without ants. Careful observations should be made in the cages to document any relationship between ants and the *Trabutina*.

e. Quantify damage caused to saltcedar by *Trabutina*. The most easily measured damage produced by *Trabutina* is likely to be dieback of infested twigs. Other, more extensive damage may be chlorosis of the foliage and wilting of tender terminals. If such damage is found, 4 to 6

terminals, each 50 cm long, should be selected and the damage quantified and recorded monthly throughout the remainder of the growing season and that of the following year. Paired undamaged terminals should be measured in a similar manner. If all terminals in the cage are damaged, then paired terminals outside the big cage may be selected.

f. Estimate populations of other arthropods in the cage. This may be estimated in the same manner as for *Diorhabda* (see Section IV.C.3.g).

g. Record damage to non-target plants inside the big cage. At each site, non-target species of importance in riparian areas may be established in the big cages for a field host-range test. These species could include willows, cottonwoods, seepwillow baccharis or other species. The plants could be planted in the soil or placed in pots inside the big cage; they must be watered frequently until well established. These species already have been tested extensively in quarantine at Temple, TX but not under field conditions.

On each monitoring date, these plants should be carefully examined visually and the numbers of each stage (adults, eggs, larvae) of *Trabutina* recorded. If developing nymphs or egg sacs are discovered on these plants, the Chairman of the Insect Monitoring Team of the Saltcedar Consortium must be notified immediately. In the history of biological control of weeds, non-target feeding by the control agents sometimes has occurred when the control agents reach high populations, then eat most of the target weed and spill over onto nearby non-target plants. This almost never continues after peak population passes but such an occurrence must immediately be

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analyzed carefully by the Insect Monitoring Team.

VI. MONITORING IN NATURE OF DISPERSAL, POPULATIONS AND BEHAVIOR OF LIBERATED CONTROL INSECTS (2ND AND 3RD YEARS)

The procedures described in this section are suggestions only and may be modified following the first year's experiences in the cages. Site personnel should make suggestions for improvements to the Insect Monitoring Team.

A. Objectives:

1. Determine establishment (development of self-sustaining, year-round populations) of control insects on growing saltcedar plants in nature.

2. Quantify reproduction, development, mortality (parasitism, predation, disease), and population increase of the control insects in nature.

3. Describe behavior [mating, oviposition, feeding by immatures and adults, and part of plant (or off-plant areas) utilized] for feeding and development of immatures and adults.

4. Determine the seasonal cycle, number of generations, overwintering.

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5. Quantify distance of dispersal of the control agents over time.

B. Method of Liberation.

A stepwise method of liberation of the control insects into nature (uncaged conditions) is recommended for two purposes, 1) to determine the effect of the natural enemies in nature (in paired caged and uncaged releases), and 2) to conserve a population of the control insects in nursery cages for additional release attempts in case the first attempts fail. If the large, overwintering cages were simply removed, allowing the control insects to disperse naturally, the control insects most probably could not be found again making monitoring uncertain; also, if they did not establish, the reasons could not easily be determined and numbers would not be available for another attempt. If permission is obtained from FWS and APHIS, the big cages should be removed in the fall after movement by *Diorhabda* has ceased, and replaced in the spring before movement begins again. This is to allow natural moisture and snow cover for the overwintering stages. If permission is not obtained, the overwintering cages should remain closed until overwintering is demonstrated and "establishment" in those cages (oviposition by overwintering adults and reproduction sufficient to maintain a population) is demonstrated in the spring.

The procedures described for the open-field monitoring may be modified after data from the year 1 releases in field cages is analyzed.

1. ***Diorhabda elongata* (leaf beetle).** The stepwise procedure for liberating the *Diorhabda*

beetles involves transferring some beetles to trees in the open, and transferring some to a new big cage to preserve a population in case the first attempt fails.

a. Transfer to uncaged trees. Transfer some eggs, larvae and/or adults to uncaged saltcedar plants in nature. A paired test should be made in which half of the control insects are placed on unbagged branches and half on branches covered by a sleeve-bag, 5 to 10 or more branches (replications) of each, each replication on a different tree. Additional control insects (if available) could be liberated on branches of other trees that are not part of the paired test. The 12 X 18" flexible insulation sheet should be placed over the sleeve-bag only, as previously done inside the big cages (see Section III.C.4).

This test is an attempt to measure reproductive rate in the field, with various predators present, that can be compared with reproduction in the cages without predators. The test may fail if the larvae disperse, or fall from the plant, and cannot be found. The effects of predation on eggs can be measured by allowing females to oviposit in sleeve-bags, then removing half the bags and retaining the other half, then counting remaining eggs over time in both treatments.

b. Transfer to another big cage. Transfer some eggs, larvae or adults as above, to saltcedar trees in another nearby large cage. A paired test of bagged and unbagged branches could be made inside the large cages as above.

c. Remove first cage. After establishment in the new large cage, the old cage may be

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removed, allowing any other control insects to disperse.

d. Stage to transfer. Transfers of *Diorhabda* should be made using eggs, larvae or adults. The large cages should be opened (if this is desired and the nursery cages are no longer needed) before the adult *Diorhabda* beetles emerge, since only the adult stage is capable of dispersal.

New sleeve bags may be established for the next generation (if desired) using adults emerged in the large cages or collected in the field.

2. *Trabutina mannipara* (mealybug). The most successful method of manual transfer that we have found to date is to transfer mature egg sacs before the neonate crawlers emerge. The procedure is to cut off twigs with the egg sacs attached and tie them to a fresh, growing terminal branch of another saltcedar plant. Care should be taken that most of the eggs have been laid inside the egg sac, because detaching the twig, and stopping the sap flow, will likely starve and kill the female. A strong colony may be established with 3 or 4 good egg sacs. Attempts to transfer crawlers after emergence from the egg sac have consistently failed in our laboratory studies, although thereotically this should be a successful method. The most probable reason for failure is that the crawlers are short lived and did not find a branch on which to feed until their energy was exhausted. To be successful, small twigs probably should be cut from the infested branch and quickly tied to a new branch. Very small twigs should be transferred so the crawlers don't have to move far to find the new branch. Liberation of *Trabutina* is very similar to that of *Diorhabda*, described above:

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a. Transfer to uncaged trees. Transfer mature egg sacs of *T. mannipara* (before the crawlers emerge) to branches of saltcedar trees, both bagged and unbagged, to establish the paired tests as for *Diorhabda*.

b. Transfer to another big cage. Transfer some egg sacs, as above, to saltcedar trees in another nearby large cage. A paired test of bagged and unbagged branches could be made inside the large cages.

c. Remove first cage. After establishment in the new large cage, the old cage may be removed, allowing any other control insects to disperse.

d. Stage to transfer. Transfers of *Trabutina* should be made using mature egg sacs before the crawlers emerge (or crawlers if this method can be developed), since the crawlers are the only stage that are mobile and able to establish on a new branch. The large cage should be opened (if this is desired and it is no longer needed as a nursery) at the same time during the life cycle, i.e. before the crawlers emerge from the egg sacs.

The sleeve bags should be opened each generation before the crawlers emerge, and new sleeve bags established by transferring egg sacs to a fresh branch and recovering with the sleeve bag, so that sufficient foliage of good quality is available for another generation.

C. Schedule of Monitoring Control Insect Dispersal and Population Increase.

Sampling frequency will depend on the occurrence of the dispersal life stages of the control insects: of adults for *Diorhabda* and of crawlers for *Trabutina*. Dispersal is projected to take place at discrete intervals, only when these stages are present, and theoretically, sampling needs to be done only during this period, or once each generation. However, in practice, discovery of the control insects in the field probably will be delayed, depending on how long the new immigrants must grow to be readily detectable.

The dispersing adults of *Diorhabda* may be easily discovered by sweeping with a net, or by collecting in light traps or by chemical attractants (however, their attractance to lights presently is unknown and no chemical attractants have yet been developed).

The dispersing crawlers of *Trabutina* probably cannot be found in nature. The first stage that can be detected probably will be the medium-sized nymphs (mid-to late 2nd instars or early 3rd instar) after they begin producing the white, waxy filaments that cover their bodies; this probably will occur 3 to 4 weeks after the nymphs have dispersed. The dates when sampling should be done can be determined by the appearance (visibility) of the nymphs still maintained in the sleeve-bags, or in other known infestations at the release site.

Initial establishment, feeding and development of the first generation outside the field cages is critical. The released eggs and/or larvae should be examined twice weekly during this period to determine establishment.

D. Sampling of Control Insects Placed in Sleeve-bags on Plants in Nature.

1. The leaf beetle, *Diorhabda elongata.* If the larvae are in nylon sleeve-bags with insulation "roofs", they should be transferred to fresh terminals and re-caged if their feeding has damaged so much foliage inside the bag that their food supply may be endangered before the next examination.

Procedures are similar to those described in Section IV.B.1 above. Twice weekly, the following counts should be made inside the sleeve-bags: a) number of eggs hatched (if eggs were placed in the cages), b) number and instar of living or dead larvae present, c) amount of feeding on the plant, d) number of other insects and spiders present, and e) plant condition. Data may be recorded on Form A1, also used for insects monitored inside the field cages.

2. The mealybug, *Trabutina mannipara.* For the paired sleeve-bagged and unbagged tests, conduct the monitoring as in Section V.D.1-6, except that monitoring of mealybugs liberated in nature will be similar to that for mealybugs in cages as described in Section V.D. and as for *Diorhabda*, paragraph VI.D.1 above.

E. Sampling Liberated, Uncaged Control Insects in Nature.

On each sampling date, careful visual surveys should be made at increasing distances away from the release site, until the limit of dispersal has been determined. These surveys should be made both upstream and downstream from the release site, or if the site is within a broad, large saltcedar infestation, then along four directions from the release site. Once the limits of

I-020130

dispersion are determined, population estimates of the control agents and the amount of damage to saltcedar should be measured at appropriate distances along the dispersal gradients.

1. The leaf beetle, *Diorhabda elongata*. These beetles may be adults released from the overwintering or secondary field cages or reared from eggs or larvae that were liberated, and the eggs, larvae and adults that develop from them in subsequent generations in the field. During the first growing season after release from the overwintering cages, conduct sampling and behavioral observations every two weeks.

a. Visual examination. Carefully examine saltcedar trees in the area near the cage and at increasing distances from the cage to determine the area in which the control insects have moved. Brief sweep samples may also be taken to determine presence or absence in an area. Adults and large larvae of *Diorhabda* will be the easiest to find. If the plants are much damaged, this may be seen first. Cast first-instar larval skins often remain attached to the plant (if not too windy) and can be seen easily if backlighted in the sunlight. The best location on the tree in which to search for the insects should be determined from the results of behavioral studies conducted in the big cage during year 1 (Section V.B.2.b above).

b. Sweep-net sampling. When the distribution of the insects is determined by the above methods, populations may be measured by shaking the saltcedar branches into a sweep net: shake 5 branches from each large tree or fewer branches from multiple smaller trees to make a total of 5 branches, and count the numbers of each stage of the beetles. This will give relative populations

I-020131

along the gradient of dispersal.

c. Examination of Plant Terminals. By this method, estimates of absolute populations can be obtained that can relate insect numbers to saltcedar foliage volume, area infested, etc. Samples can be taken by:

Counting the numbers of each stage of the beetles per 50 cm of terminal branch. Samples may be taken from top and lateral terminals on each tree, on 2 to 4 trees at each distance interval, at distance intervals such as 20, 50, 100, 500 m etc. from the release site, both upstream and downstream, or along transects in 4 directions within a large, broad infestation. If the results from year 1 in the big cages indicate a preference of the beetles for a part of the tree (Section V.B.2.b above) the samples should consistently be from the same part of the tree to avoid biasing the data.

2. The mealybug, *Trabutina mannipara*. These mealybugs may be egg sacs released from the overwintering or secondary field cages or reared from egg-sacs that were liberated, and nymphs or egg sacs that develop from them in subsequent generations in nature.

During the first growing season after release from the overwintering cages, conduct sampling and behavioral observations every two weeks. Carefully examine saltcedar trees in the area near the cage and at increasing distance from the cage to determine the area in which the mealybugs have moved. The white masses of nymphs and the egg sacs should be readily visible on the plants.

I-020132

VII. MONITORING EFFECTS ON VEGETATION

A. Damage to Saltcedar in Nature.

Damage caused by the control insects should be quantified in nature along the same dispersal gradients where control-insect populations are monitored. Damage evaluation of saltcedar is particularly difficult because of the growth form of the plant foliage, making the direct measurement of the amount of feeding virtually impossible. Also, the measurement of feeding is insufficient for measuring damage. Observations of heavily damaged plants in Turkmenistan, Kazakhstan and China reveal that, in addition to the foliage consumed, much foliage had died and remained hanging on the plant, apparently the result of the beetles feeding on small stems which had then caused the death of the foliage distad of that point.

1. Direct measurement of foliage consumed and killed. In practice, this is very difficult to measure.

2. Visual assignment to damage categories. This method will depend on the skill of the person making the assignments in the field, and the use of guideline photographs obtained from the field cages during year 1 (which are likely to be influenced by leafhopper damage). Evaluation will depend on the visual summation of the foliage consumed, and the foliage killed but remaining on the plant or already fallen off, compared with healthy foliage remaining on the plant. Suggested categories are: no apparent damage, 5-10%, 10-35%, 35-70%, 70-90%, 90-100% damage.

I-020133

3. Dieback of branches. Select trees of different sizes at different distances from the release site and measure the length of dead branches on each tree. Sample 30 small plants (1 to 4 ft high), 10 medium-sized plants (6 to 10 ft high, 3 to 6 ft canopy diameter) and 3 large plants (10 to 25 ft high and 10 to 30 ft canopy diameter) at each distance from the release site. Measure the same trees repeatedly, in June and September of each year.

4. Reduction in density and size of living trees. Establish three permanent transects, each 3 m wide and 50 m long at different distances from the release site. Measure height, number and size of stems, and canopy diameter of each saltcedar plant along the transect. Locate transects with a variety of sizes of saltcedar plants, rather than locating them randomly. The transects at the release site will be established and counted at the end of the first year (before insects are released from the big cages) to obtain baseline data, and annually in June thereafter. Location of more distant transects will be determined from the data on the dispersal of the control agents.

5. Remote sensing. During the first or second year, baseline remote sensing data will be obtained at each site, by high resolution, 9" aerial photography film, flown from 5,000 ft, with the release cage located in the center. This resolution can detect the size and shape of individual medium-sized trees. The sites should be flown in late fall, when the saltcedar trees have turned a golden-orange color just before leaf fall. Re-flights will be made when damage and expansion of damaged areas becomes apparent from the ground surveys.

I-020134

B. Damage to Non-target Vegetation in Nature.

Damage to non-target plants in nature is not expected, based on laboratory tests performed during the quarantine studies, testing and surveys done overseas, and literature and museum records. The additional data from the big sleeve cages at each release site, under very high population pressure to feed and oviposit, also should be evaluated. The proof of all these observations and experiments will lie in whether the control insects oviposit, feed, or complete their development on non-target plants in nature, uninfluenced by any previous cage artifacts that sometimes predict feeding that does not occur in nature.

1. Visual examination. Non-target plants should be examined visually in areas near the release site and in areas into which the control insects have dispersed, especially in areas where control insect populations on saltcedar are high. These examinations should search for adults, eggs, larvae and feeding damage on the plants. Adults on a plant means little unless feeding and oviposition are occurring, and feeding alone means little. Oviposition also means little unless the larvae feed and complete their development on non-target plants.

VIII. CLIMATE AND PHYSICAL ENVIRONMENT MONITORING

Most of the parameters to be monitored that relate to the control insects and their effect on saltcedar, will be strongly influenced by the physical environment. These include whether or not the control insects become established, their survival, rate of development, number of generations, seasonal occurrence, behavior, dispersal rate, and amount of damage to saltcedar. For example, preliminary information indicates that high relative humidity or excessive moisture

may be harmful to the *Diorhabda* inside the sleeve-bags and may favor the leafhoppers inside the big cages. Flooding may drown *Diorhabda* pupae and excessively high or low temperatures may limit control insect establishment and/or population levels. Some of these physical factors are scheduled for monitoring by the Abiotic Factors Monitoring Team. However, since a knowledge of these factors is essential to the Insect Monitoring Program, monitoring of some of these factors (temperature, humidity, rainfall) are included here. Other factors (soil type, soil salinity) should be measured at the beginning of the insect program. All data on these abiotic factors will be shared between all the monitoring programs. These abiotic factors should be monitored as follows:

A. Temperature and Humidity.

These parameters should be monitored continuously inside the sleeve-bags, inside the big cages but outside of the sleeve-bags, and outside the big cages. Monitoring should be with an automatic data logger such as the "Hobo" brand, set to record each 15 min. These recorders should be shielded from direct sunlight underneath the sleeve-bag insulation board shields and under a similar shield outside the cage. Data should be downloaded at each monitoring date so it will not be lost in case the recorder malfunctions or is damaged or stolen.

B. Precipitation.

A continuously recording gauge to measure rainfall and snowfall should be established at each site.

I-020136

C. Soil Type.

Soil type should be determined at each site, to include percent sand, clay, etc. as well as organic matter and nutrients. This should be measured at the 0-2 inch and at the 12 inch levels.

D. Salinity: Soil and Groundwater.

Salinity of the surface soil may influence pupation by *Diorhabda*, and should be analyzed at the beginning of the monitoring program. Information on groundwater salinity should be obtained from the Abiotic Factor Monitoring Team.

E. Depth to Groundwater.

This factor should be measured monthly to obtain seasonal variation between the wettest and the driest times of year. Hourly diurnal variation also may be recorded at critical times during the year. This monitoring will require recording gauges in wells and should be performed by the Abiotic Factor Monitoring Team.

IX. PLAN FOR SUPPRESSION OR ELIMINATION OF CONTROL INSECTS IF EFFECTS ARE DETRIMENTAL

Both the 28 August "Proposal to Fish and Wildlife Service" and the Environmental Assessment require a contingency plan for the use of insecticides to suppress or eliminate the control insects if they cause or threaten harm to the ecosystem.

I-020137

A. Criteria for Declaring Effects to be Detrimental.

1. Research data reveals that the control insects are able to complete their life cycle and reproduce in sufficient numbers to damage non-target native species or other moderate or highly beneficial plants.

2. The control insects quickly kill saltcedar trees at the release sites and give indications of rapid movement away from the sites.

B. Action to be Taken.

Immediate notification will be forwarded to the Chairman of the Saltcedar Consortium, who will immediately notify the Chairman of USDA-APHIS-TAGIBCAW, and to appropriate personnel of USDI Fish and Wildlife Service.

1. If the control insects cause important damage to non-target plants, the Saltcedar Consortium and the concerned Agencies will consider, and require if justified, that applications of insecticides and/or other appropriate controls be made to eradicate the control insects at all sites. It is important that such action be initiated quickly, before the control insects disperse beyond the area where control is possible.

2. If the control insects quickly kill saltcedar trees at the release sites and give indications of rapid dispersal away from the sites, the Saltcedar Consortium and the concerned agencies will consider, and require if justified, that insecticides and/or other appropriate controls be applied in

sub-lethal doses to slow the effects or dispersal to acceptable levels. This, in itself, will include various research treatments to determine proper materials, dosages and methods.

APPENDIX A
Photographs and Sketches

Figure 1. Saltcedar leaf beetle adults and 1st and 3rd instar larvae feeding on *Tamarix*.

I-020140

Figure 2A-C. The manna mealybug, *Trabutina mannipara*. A) Colony of adult mealybugs on *Tamarix*. B) Close-up of two adult females exuding honeydew from within ovisacs. C) Nymphs of *T. mannipara* developing on *Tamarix*.

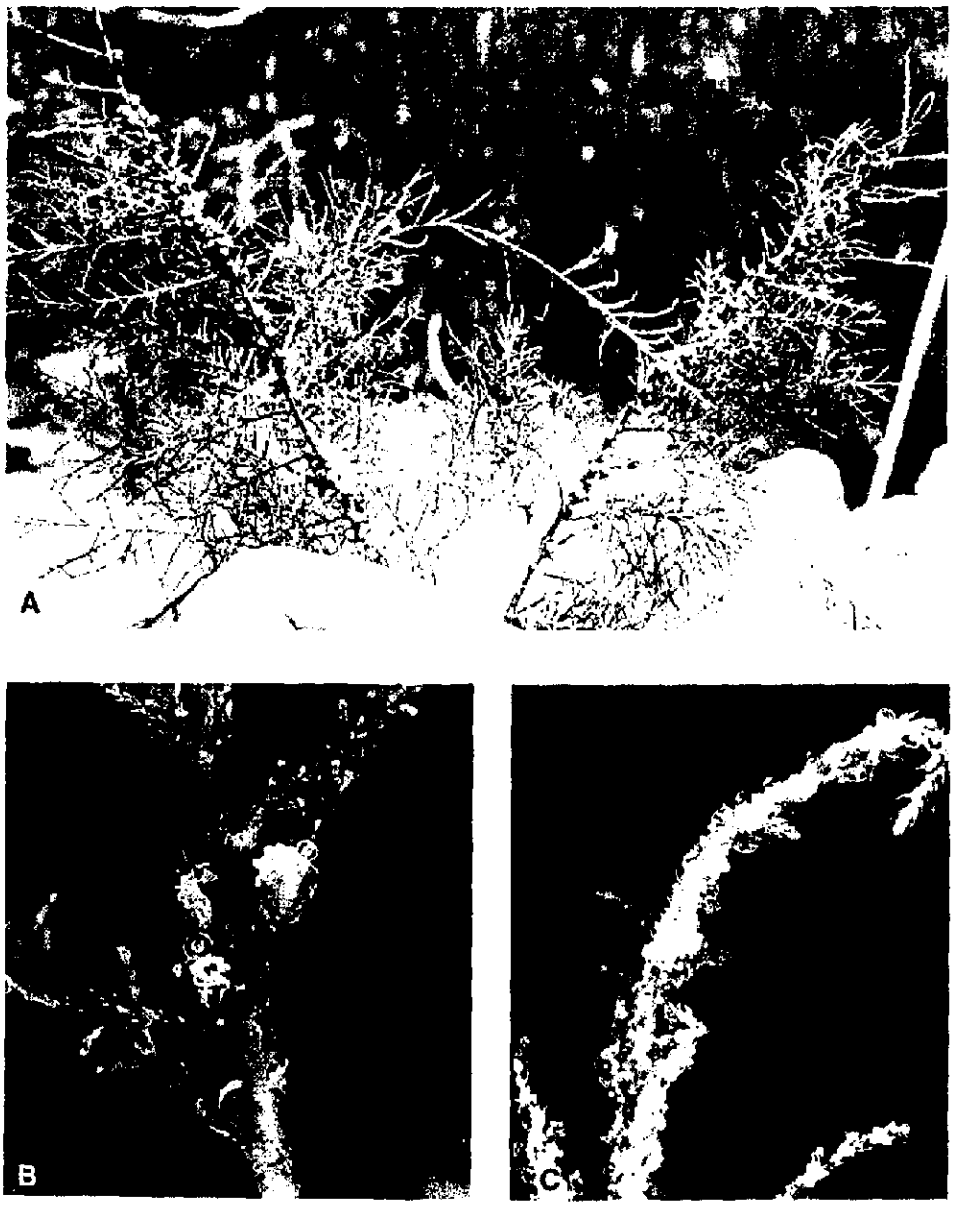

Figure 3. Field cage with gated fence at Pueblo, Colorado.

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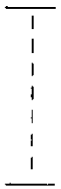
Figure 4. Sleeve cage for monitoring insect development.

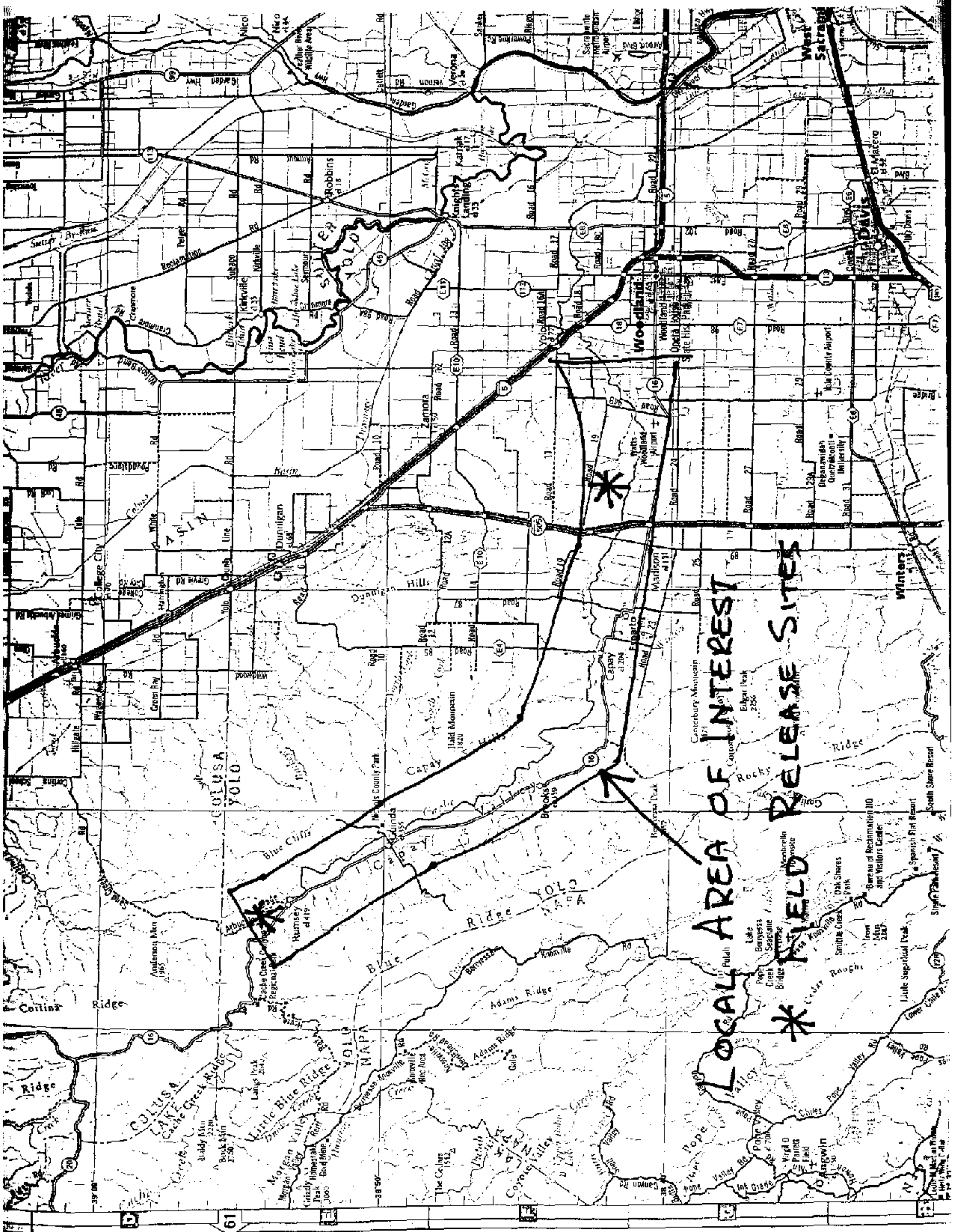


1. Cut center out of 2 liter coke bottle (5"). Make sure to cut off the rounded portions and the top and bottom – if a rim is left the plastic will collapse.

2. Cut two pieces of organdy (12"x13") and fashion them into cylinders using a sewing machine with a fine stitch length or glue them together with DAP (silicone type product that sets fast.

3. Attach the organdy cylinders to each side of the plastic cylinder using DAP or similar product. Hot glue does not work (melts in the sun!)

Figure 5. Protective roofs for sleeve bags to protect small larvae from rain.



APPENDIX J

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APPENDIX D

USDA

United States Department of Agriculture
Research, Education and Economics
Agricultural Research Service

April 13, 1999

Mr. David Morrison
Resource Manager
Planning and Public Works Department
Yolo County
292 W. Beamer St.
Woodland, CA 95695

Dear Mr. Morrison:

The US Department of Agriculture, Agricultural Research Service is working with a number of different public and private groups to develop new methods to control invasive plant species such as saltcedar and giant reed. Both of these species are invading the Cache Creek riparian area in your county. We are currently working with the Cache Creek Conservancy, Team *Arundo* del Norte, the US Fish and Wildlife Service and the University of California in evaluating the use of biological control to help reduce the impact and spread of these detrimental plants throughout the state of California and in other states in the west. We have been in communication with others in your department about this project but now want to formally announce our intention to submit a CalFed proposal to acquire funding to implement a research and implementation project for this effort that specifically addresses funding for a program along Cache Creek.

Currently, I have an appointment to meet with some of your staff next week out in the Woodland office in conjunction with Jan Lowery of the Cache Creek Conservancy. I also plan to provide an overview presentation to the entire membership of the Cache Creek Conservancy at their May 24 meeting. I would like to personally invite you to attend either, or both of these meetings to find out more about our proposed program. You may also contact me directly [(510) 559-6127) at any time for more detailed information. Once completed, I will be forwarding you a copy of our CalFed proposal.

Thank you very much for you time and interest in our project. Hopefully, this effort will be successful and we will be able to save you great time and expense in reducing these pest plant populations to non-significant problems.

Sincerely,

Raymond I. Carruthers
Research Leader

cc County Board of Supervisors

Pacific West Area - Western Regional Research Center
Exotic and Invasive Weeds Research
800 Buchanan Street • Albany, CA 94710-1105
Voice: 510 559-6127 • Fax: 510 559-6123 • E-mail: ric@pw.usda.gov

Agricultural Research - Investing in Your Future

I-020145

USDA

United States Department of Agriculture
Research, Education and Economics
Agricultural Research Service

April 13, 1999

Delta Protection Commission
14215 River Road
PO Box 530
Walnut Grove, CA 95690

Dear Sirs:

The US Department of Agriculture, Agricultural Research Service is working with a number of different public and private groups to develop new methods to control invasive plant species such as saltcedar and giant reed. Both of these species are invading the Cache Creek riparian area in your area. We are currently working with the Cache Creek Conservancy, Team *Arundo* del Norte, the US Fish and Wildlife Service and the University of California in evaluating the use of biological control to help reduce the impact and spread of these detrimental plants throughout the state of California and in other states in the west. We have been in communication with Yolo County Planning and Public Works Department about this project but now want to formally announce our intention to you in regard to your submission of a CalFed proposal to acquire funding to implement a research and implementation project for this effort that specifically addresses Cache Creek.

Currently, I have an appointment to meet with some of the County staff next week out in their Woodland office in conjunction with Jan Lowery of the Cache Creek Conservancy. I also plan to provide an overview presentation to the entire membership of the Cache Creek Conservancy at their May 24 meeting. I would like to personally invite you to attend either, or both of these meetings to find out more about our proposed program. You may also contact me directly [(510) 559-6127) at any time for more detailed information. Once completed, I will be forwarding you a copy of our CalFed proposal.

Thank you very much for you time and interest in our project. Hopefully, this effort will be successful and we will be able to save the County and local landowners great time and expense in reducing these pest plant populations to non-significant problems.

Sincerely,

Raymond I. Carruthers
Research Leader

ars

Pacific West Area - Western Regional Research Center
Exotic and Invasive Weeds Research
800 Buchanan Street • Albany, CA 94710-1105
Voice: 510 559-6127 • Fax: 510 559-6123 • E-mail: rc@pw.usda.gov

Agricultural Research - Investing in Your Future

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Agreement No. _____

Exhibit _____

STANDARD CLAUSES - R. Parrott
SERVICE & CONSULTANT SERVICE CONTRACTS FOR $5,000 & OVER WITH NONPUBLIC ENTITIES

Workers' Compensation Clause. Contractor affirms that it is aware of the provisions of Section 3700 of the California Labor Code which require every employer to be insured against liability for workers' compensation or to undertake self-insurance in accordance with the provisions of that Code, and Contractor affirms that it will comply with such provisions before commencing the performance of the work under this contract.

National Labor Relations Board Clause. In accordance with Public Contract Code Section 10296, Contractor declares under penalty of perjury that no more than one final, unappealable finding of contempt of court by a federal court has been issued against the Contractor within the immediately preceding two-year period because of Contractor's failure to comply with an order of a federal court which orders Contractor to comply with an order of the national Labor Relations Board.

Nondiscrimination Clause. During the performance of this contract, the recipient,Ccontractor and its subcontractors shall not deny the contract's benefits to any person on the basis of religion, color, ethnic group identification, sex, age, physical or mental disability, nor shall they discriminate unlawfully against any employee or applicant for employment because of race, religion, color, national origin, ancestry, physical handicap, mental disability, medical condition, marital status, age (over 40), or sex. Contractor shall insure that the evaluation and treatment of employees and applicants for employment are free of such discrimination. Contractor shall comply with the provisions of the Fair Employment and Housing Act (Government Code Section 12900 et seq.), the regulations promulgated thereunder (California Administrative Code, Title 2, Sections 7285.0 et seq.), the provisions of Article 9.5, Chapter 1, Part 1, Division 3, Title 2 of the Government Code (Government Code Sections 11135 - 11139.5), and the regulations or standards adopted by the awarding State agency to implement such article. Contractor or recipient shall permit access by representatives of the Department of Fair Employment and Housing and the awarding State agency upon reasonable notice at any time during the normal business hours, but in no case less than 24 hours' notice, to such of its books, records, accounts, other sources of information and its facilities as said Department or Agency shall require to ascertain compliance with this clause. Recipient, Contractor and its subcontractors shall give written notice of their obligations under this clause to labor organizations with which they have a collective bargaining or other agreement. The Contractor shall include the nondiscrimination and compliance provisions of this clause in all subcontracts to perform work under the contract.

Statement of Compliance. The Contractor's signature affixed hereon and dated shall constitute a certification under penalty of perjury under the laws of the State of California that the Contractor has, unless exempted, complied with the nondiscrimination program requirements of Government Code Section 12990 and Title 2, California Code of Regulations, Section 8103.

Performance Evaluation. For consulting service agreements, Contractor's performance under this contract will be evaluated after completion. A negative evaluation will be filed with the Department of General Services.

Availability of Funds. Work to be performed under this contract is subject to availability of funds through the State's normal budget process.

Audit Clause. For contracts in excess of $10,000, the contracting parties shall be subject to the examination and audit of the State Auditor for a period of three years after final payment under the contract. (Government Code Section 8546.7).

Payment Retention Clause. Ten percent of any progress payments that may be provided for under this contract shall be withheld per Public Contract Code Sections 10346 and 10379 pending satisfactory completion of all services under the contract.

Reimbursement Clause. If applicable, travel and per diem expenses to be reimbursed under this contract shall be at the same rates the State provides for unrepresented employees in accordance with the provisions of Title 2, Chapter 3, of the California Code of Regulations. Contractor's designated headquarters for the purpose of computing such expenses shall be: _____.

Disabled Veteran Business Enterprise Participation Requirement Audit Clause. Contractor or vendor agrees that the awarding department or its delegates will have the right to review, obtain, and copy all records pertaining to performance of the contract. Contractor or vendor agrees to provide the awarding department or its delegatee access to its premises, upon reasonable notice, during normal business hours for the purpose of interviewing employees and inspecting and copying such books, records, accounts, and other material that may be relevant to a matter under investigation for the purpose of determining compliance with Public Contract Code Section 10115 et seq. Contractor or vendor further agrees to maintain such records for a period of three (3) years after final payment under the contract. Title 2 CCR Section 1896.75.

Priority Hiring Considerations. For contracts in excess of $200,000, the Contractor shall give priority consideration in filling vacancies in positions funded by the contract to qualified recipients of aid under Welfare and Institutions Code Section 11200. (Public Contract Code Section 10353).

Drug-Free Workplace Certification. By signing this contract, the Contractor or grantee hereby certifies under penalty of perjury under the laws of the State of California that the Contractor or grantee will comply with the requirements of the Drug-Free Workplace Act of 1990 (Government Code Section 8350 et seq.) and will provide a drug-free workplace by taking the following actions:

1. Publish a statement notifying employees that unlawful manufacture, distribution, dispensation, possession, or use of a controlled substance is prohibited and specifying actions to be taken against employees for violations.

2. Establish a Drug-Free Awareness Program to inform employees about all of the following:

   (a) The dangers of drug abuse in the workplace,
   (b) The person's or organization's policy of maintaining a drug-free workplace,
   (c) Any available counseling, rehabilitation and employee assistance programs, and
   (d) Penalties that may be imposed upon employees for drug abuse violations.

3. Every employee who works on the proposed contract or grant:

   (a) Will receive a copy of the company's drug-free policy statement, and
   (b) Will agree to abide by terms of the company's statement as a condition of employment on the contract or grant.

This contract or grant may be subject to suspension of payments or termination, or both, and the Contractor or grantee may be subject to debarment if the department determines that: (1) the Contractor or grantee has made a false certification, or (2) the Contractor or grantee violates the certification by failing to carry out the requirements noted above.

Antitrust Claims. In submitting a bid to a public purchasing body, the bidder offers and agrees that if the bid is accepted, it will assign to the purchasing body all rights, title, and interest in and to all causes of action it may have under Section 4 of the Clayton Act (15 U.S.C. Sec. 15) or under the Cartwright Act (Chapter 2 (commencing with Section 16700) Part 2 of Division 7 of the Business and Professions Code), arising from purchases of goods, materials, or services by the bidder for sale to the purchasing body pursuant to the bid. Such assignment shall be made and become effective at the time the purchasing body tenders final payment to the bidder. See Government Code Section 4552.

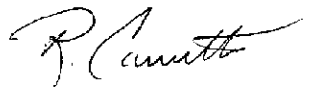
If an awarding body or public purchasing body received, either through judgment or settlement, a monetary recovery for a cause of action assigned under this chapter, the assignor shall be entitled to receive reimbursement for actual legal costs incurred and may, upon demand, recover from the public body any portion of the recovery, including treble damages, attributable to overcharges that were paid by the assignor but were not paid by the public body as part of the bid price, less the expenses incurred in obtaining that portion of the recovery. See Government Code Section 4553.

Upon demand in writing by the assignor, the assignee shall, within one year from such demand, reassign the cause of action assigned under this part if the assignor has been or may have been injured by the violation of law for which the cause of action arose and (a) the assignee has not been injured thereby, or (b) the assignee declines to file a court action for the cause of action. See Government Code Section 4554.

Americans With Disabilities Act. By signing this contract, Contractor assures the state that it complies with the Americans With Disabilities Act (ADA) of 1990, (42 U.S.C. 12101 et seq.), which prohibits discrimination on the basis of disability, as well as all applicable regulations and guidelines issued pursuant to the ADA.

Corporate Qualifications To Do Business in California. Contractor must be currently qualified to do business in California as defined by the Revenue & Taxation Code, Section 23101 unless exempted. Both domestic and foreign corporations (those incorporated outside of California) must be in good standing in order to be qualified to do business in California.

Former State Employees: a) For the two-year period from the date he or she left State employment, no former State officer or employee may enter into a contract in which he or she engaged in any of the negotiations, transactions, planning, arrangements or any part of the decision-making process relevant to the contract while employed in any capacity by any State agency. b) For the twelve-month period from the date he or she left State employment, no former State officer or employee may enter into a contract with any State agency if he or she was employed by that State agency in a policy-making position in the same general subject area as the proposed contract within the twelve-month period prior to his or her leaving State service.



DWR 4099 (Rev. 9/95) SIDE B

Agreement No._____

Exibit _____

ADDITIONAL STANDARD CLAUSES

Recycled Materials. Contractor hereby certifies under penalty of perjury that ____ (enter value or "0" here) percent of the materials, goods and supplies offered or products used in the performance of this Agreement meets or exceeds the minimum percentage of recycled material as defined in Sections 12161 and 12200 of the Public Contract Code.

Severability. If any provision of this Agreement is held invalid or unenforceable by any court of final jurisdiction, it is the intent of the parties that all other provisions of this Agreement be construed to remain fully valid, enforceable, and binding on the parties.

Governing Law. This Agreement is governed by and shall be interpreted in accordance with the laws of the State of California.

Y2K Language. The Contractor warrants and represents that the goods or services sold, leased, or licensed to the State of California, its agencies, or its political subdivisions, pursuant to this Agreement are "Year 2000 compliant." For purposes of this Agreement a good or service is Year 2000 compliant if it will continue to fully function before, at, and after the Year 2000 without interruption and, if applicable, with full ability to accurately and unambiguously process, display, compare, calculate, manipulate, and otherwise utilize date information. This warranty and representation supersedes all warranty disclaimers and limitations and all limitations on liability provided by or through the Contractor.

Child Support Compliance Act. For any Agreement in excess of $100,000, the Contractor acknowledges in accordance therewith, that:

1. The Contractor recognizes the importance of child and family support obligations and shall fully comply with all applicable state and federal laws relating to child and family support enforcement, including, but not limited to, disclosure of information and compliance with earnings assignment orders, as provided in Chapter 8 (commencing with Section 5200) of Part 5 of Division 9 of the Family Code; and

2. The Contractor, to the best of its knowledge, is fully complying with the earnings assignment orders of all employees and is providing the names of all new employees to the New Hire Registry maintained by the California Employment Development Department.

U.S. Department of the Interior

Certifications Regarding Debarment, Suspension and Other Responsibility Matters, Drug-Free Workplace Requirements and Lobbying

Persons signing this form should refer to the regulations referenced below for complete instructions:

Certification Regarding Debarment, Suspension, and Other Responsibility Matters - Primary Covered Transactions - The prospective primary participant further agrees by submitting this proposal that it will include the clause titled, "Certification Regarding Debarment, Suspension, Ineligibility and Voluntary Exclusion - Lower Tier Covered Transaction," provided by the department or agency entering into this covered transaction, without modification, in all lower tier covered transactions and in all solicitations for lower tier covered transactions. See below for language to be used; use this form for certification and sign; or use Department of the Interior Form 1954 (DI-1954). (See Appendix A of Subpart D of 43 CFR Part 12.)

Certification Regarding Debarment, Suspension, Ineligibility and Voluntary Exclusion - Lower Tier Covered Transactions - (See Appendix B of Subpart D of 43 CFR Part 12.)

Certification Regarding Drug-Free Workplace Requirements - Alternate I. (Grantees Other Than Individuals) and Alternate II. (Grantees Who are Individuals) - (See Appendix C of Subpart D of 43 CFR Part 12)

Signature on this form provides for compliance with certification requirements under 43 CFR Parts 12 and 18. The certifications shall be treated as a material representation of fact upon which reliance will be placed when the Department of the Interior determines to award the covered transaction, grant, cooperative agreement or loan.

PART A: Certification Regarding Debarment, Suspension, and Other Responsibility Matters - Primary Covered Transactions

---

CHECK ✓ IF THIS CERTIFICATION IS FOR A PRIMARY COVERED TRANSACTION AND IS APPLICABLE.

(1) The prospective primary participant certifies to the best of its knowledge and belief, that it and its principals:

(a) Are not presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from covered transactions by any Federal department or agency;

(b) Have not within a three-year period preceding this proposal been convicted of or had a civil judgment rendered against them for commission of fraud or a criminal offense in connection with obtaining, attempting to obtain, or performing a public (Federal, State or local) transaction or contract under a public transaction; violation of Federal or State antitrust statutes or commission of embezzlement, theft, forgery, bribery, falsification or destruction of records, making false statements, or receiving stolen property;

(c) Are not presently indicted for or otherwise criminally or civilly charged by a governmental entity (Federal, State or local) with commission of any of the offenses enumerated in paragraph (1)(b) of this certification; and

(d) Have not within a three-year period preceding this application/proposal had one or more public transactions (Federal, State or local) terminated for cause or default.

(2) Where the prospective primary participant is unable to certify to any of the statements in this certification, such prospective participant shall attach an explanation to this proposal.

PART B: Certification Regarding Debarment, Suspension, Ineligibility and Voluntary Exclusion - Lower Tier Covered Transactions

---

CHECK__ IF THIS CERTIFICATION IS FOR A LOWER TIER COVERED TRANSACTION AND IS APPLICABLE

(1) The prospective lower tier participant certifies, by submission of this proposal, that neither it nor its principals is presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from participation in this transaction by any Federal department or agency.

(2) Where the prospective lower tier participant is unable to certify to any of the statements in this certification, such prospective participant shall attach an explanation to this proposal.

DI-2010
March 1990
(This form supersedes DI-1953, DI-1954, DI-1955, DI-1956 and DI-1962)

PART C: Certification Regarding Drug-Free Workplace Requirements

CHECK__ IF THIS CERTIFICATION IS FOR AN APPLICANT WHO IS NOT AN INDIVIDUAL.

Alternate I. (Grantees Other Than Individuals)

A. The grantee certifies that it will or continue to provide a drug-free workplace by:

(a) Publishing a statement notifying employees that the unlawful manufacture, distribution, dispensing, possession, or use of a controlled substance is prohibited in the grantee's workplace and specifying the actions that will be taken against employees for violation of such prohibition;

(b) Establishing an ongoing drug-free awareness program to inform employees about--
(1) The dangers of drug abuse in the workplace;
(2) The grantee's policy of maintaining a drug-free workplace;
(3) Any available drug counseling, rehabilitation, and employee assistance programs; and
(4) The penalties that may be imposed upon employees for drug abuse violations occurring in the workplace;

(c) Making it a requirement that each employee to be engaged in the performance of the grant be given a copy of the statement required by paragraph (a);

(d) Notifying the employee in the statement required by paragraph (a) that, as a condition of employment under the grant, the employee will --
(1) Abide by the terms of the statement; and
(2) Notify the employer in writing of his or her conviction for a violation of a criminal drug statute occurring in the workplace no later than five calendar days after such conviction;

(e) Notifying the agency in writing, within ten calendar days after receiving notice under subparagraph (d)(2) from an employee or otherwise receiving actual notice of such conviction. Employers of convicted employees must provide notice, including position title, to every grant officer on whose grant activity the convicted employee was working, unless the Federal agency has designated a central point for the receipt of such notices. Notice shall include the identification numbers(s) of each affected grant;

(f) Taking one of the following actions, within 30 calendar days of receiving notice under subparagraph (d)(2), with respect to any employee who is so convicted --
(1) Taking appropriate personnel action against such an employee, up to and including termination, consistent with the requirements of the Rehabilitation Act of 1973, as amended; or
(2) Requiring such employee to participate satisfactorily in a drug abuse assistance or rehabilitation program approved for such purposes by a Federal, State, or local health, law enforcement, or other appropriate agency;

(g) Making a good faith effort to continue to maintain a drug-free workplace through implementation of paragraphs (a) (b), (c), (d), (e) and (f).

B. The grantee may insert in the space provided below the site(s for the performance of work done in connection with the specific grant:

Place of Performance (Street address, city, county, state, zip code)
USDA - ARS
800 BUCHANAN ST.
ALBANY, CA 94710

Check___if there are workplaces on file that are not identified here.

PART D: Certification Regarding Drug-Free Workplace Requirements

CHECK__ IF THIS CERTIFICATION IS FOR AN APPLICANT WHO IS AN INDIVIDUAL.

Alternate II. (Grantees Who Are Individuals)

(a) The grantee certifies that, as a condition of the grant, he or she will not engage in the unlawful manufacture, distribution, dispensing, possession, or use of a controlled substance in conducting any activity with the grant;

(b) If convicted of a criminal drug offense resulting from a violation occurring during the conduct of any grant activity, he or she will report the conviction, in writing, within 10 calendar days of the conviction, to the grant officer or other designee, unless the Federal agency designates a central point for the receipt of such notices. When notice is made to such a central point, it shall include the identification number(s) of each affected grant

I-020151

PART E: Certification Regarding Lobbying
Certification for Contracts, Grants, Loans, and Cooperative Agreements

CHECK__ IF CERTIFICATION IS FOR THE AWARD OF ANY OF THE FOLLOWING AND THE AMOUNT EXCEEDS $100,000: A FEDERAL GRANT OR COOPERATIVE AGREEMENT; SUBCONTRACT, OR SUBGRANT UNDER THE GRANT OR COOPERATIVE AGREEMENT.

CHECK__ IF CERTIFICATION IS FOR THE AWARD OF A FEDERAL LOAN EXCEEDING THE AMOUNT OF $150,000, OR A SUBGRANT OR SUBCONTRACT EXCEEDING $100,000, UNDER THE LOAN.

The undersigned certifies, to the best of his or her knowledge and belief, that:

(1) No Federal appropriated funds have been paid or will be paid, by or on behalf of the undersigned, to any person for influencing or attempting to influence an officer or employee of an agency, a Member of Congress, and officer or employee of Congress, or an employee of a Member of Congress in connection with the awarding of any Federal contract, the making of any Federal grant, the making of any Federal loan, the entering into of any cooperative agreement, and the extension, continuation, renewal, amendment, or modification of any Federal contract, grant, loan, or cooperative agreement.

2) If any funds other than Federal appropriated funds have been paid or will be paid to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with this Federal contract, grant, loan, or cooperative agreement, the undersigned shall complete and submit Standard Form-LLL, "Disclosure Form to Report Lobbying," in accordance with its instructions.

3) The undersigned shall require that the language of this certification be included in the award documents for all subawards at all tiers (including subcontracts, subgrants, and contracts under grants, loans, and cooperative agreements) and that all subrecipients shall certify accordingly.

his certification is a material representation of fact upon which reliance was placed when this transaction was made or entered to. Submission of this certification is a prerequisite for making or entering into this transaction imposed by Section 1352, title 1, U.S. Code. Any person who fails to file the required certification shall be subject to a civil penalty of not less than $10,000 id not more than $100,000 for each such failure.

: the authorized certifying official, I hereby certify that the above specified certifications are true.

GNATURE OF AUTHORIZED CERTIFYING OFFICIAL R. Carruthers

PED NAME AND TITLE RAYMOND I. CARRUTHERS, RESEARCH LEADER

TE APRIL 14, 1999

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FEDERAL ASSISTANCE

2. DATE SUBMITTED	Applicant Identifier
04/16/99	

1. TYPE OF SUBMISSION:		3. DATE RECEIVED BY STATE	State Application Identifier
Application ☐ Construction ☒ Non-Construction	Preapplication ☐ Construction ☐ Non-Construction	4. DATE RECEIVED BY FEDERAL AGENCY	Federal Identifier

5. APPLICANT INFORMATION

Legal Name:	Organizational Unit:
Raymond I. Carruthers	USDA-ARS - Exotic and Invasive Weeds
Address *(give city, county, State, and zip code):*	Name and telephone number of person to be contacted on matters involving this application *(give area code)*
USDA-ARS 800 Buchanan St Albany, CA 94710 Alameda Co	510 559-6127

6. EMPLOYER IDENTIFICATION NUMBER *(EIN):*
94-1610000

7. TYPE OF APPLICANT: *(enter appropriate letter in box)* ☐

A. State — H. Independent School Dist.
B. County — I. State Controlled Institution of Higher Learning
C. Municipal — J. Private University
D. Township — K. Indian Tribe
E. Interstate — L. Individual
F. Intermunicipal — M. Profit Organization
G. Special District — N. Other (Specify) Federal Govt.

8. TYPE OF APPLICATION:
☒ New ☐ Continuation ☐ Revision

If Revision, enter appropriate letter(s) in box(es) ☐ ☐

A. Increase Award B. Decrease Award C. Increase Duration
D. Decrease Duration Other *(specify)*:

9. NAME OF FEDERAL AGENCY:
US Dept. Agric.
Agricultural Research Service

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER:
☐☐-☐☐☐

TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:

12. AREAS AFFECTED BY PROJECT *(Cities, Counties, States, etc.):*
Yolo County, CA

13. PROPOSED PROJECT

Start Date	Ending Date
10/1/99	9/30/03

14. CONGRESSIONAL DISTRICTS OF:

a. Applicant	b. Project

15. ESTIMATED FUNDING:

Federal	$ 1,042,885 .00
Applicant	$ .00
State	$ .00
Local	$ .00
Other	$ .00
Program Income	$ .00
TOTAL	$ 1,042,885 .00

16. IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?

a. YES. THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE _____

b. No. ☑ PROGRAM IS NOT COVERED BY E. O. 12372
☐ OR PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. IS THE APPLICANT DELINQUENT ON ANY FEDERAL DEBT?
☐ Yes If "Yes," attach an explanation. ☑ No

18. TO THE BEST OF MY KNOWLEDGE AND BELIEF, ALL DATA IN THIS APPLICATION/PREAPPLICATION ARE TRUE AND CORRECT, THE DOCUMENT HAS BEEN DULY AUTHORIZED BY THE GOVERNING BODY OF THE APPLICANT AND THE APPLICANT WILL COMPLY WITH THE ATTACHED ASSURANCES IF THE ASSISTANCE IS AWARDED.

a. Type Name of Authorized Representative	b. Title	c. Telephone Number
Raymond I. Carruthers	Research Leader	510 559-6127

d. Signature of Authorized Representative

e. Date Signed: April 15, 99

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